PARASITOLOGY

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PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

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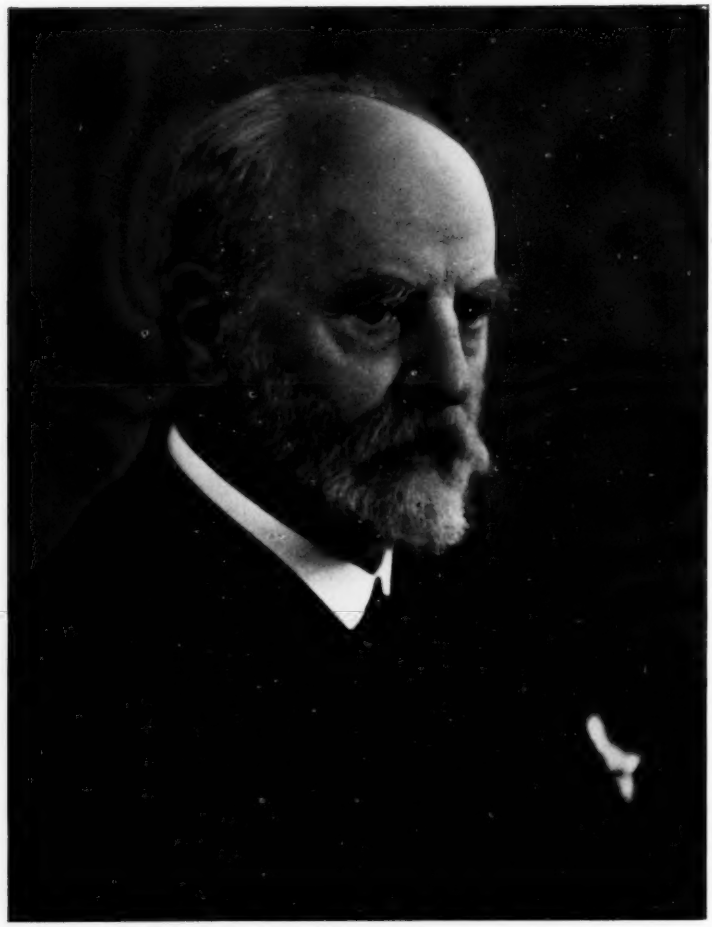

CECIL WARBURTON, M.A. (CANTAB.)

On February 6, 1934, our honoured colleague and friend, Mr Warburton, attained his eightieth year, and we mark the occasion by publishing his recently taken portrait as a frontispiece to this volume.

UNIV. OF MICH.

VOLUME XXVI APRIL, 1934 No. 1

A FUNGUS DISEASE IN FISHES OF THE GULF OF MAINE[1]

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Associate Aquatic Biologist, United States Bureau of Fisheries

(With Plate I, containing Figs. 1–5, and Figs. I and II in the Text)

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I. INTRODUCTION

DURING 1931, a co-operative investigation of a disease reported from marine fishes in the Gulf of Maine was undertaken by the United States Bureau of Fisheries and the State of Maine. This investigation, started at Eastport, Maine, and later transferred to Boothbay Harbour, Maine, where more suitable facilities were found at the Bureau of Fisheries Station, was terminated in October of that year when climatic conditions rendered it impossible to carry on field investigations. Subsequent to 1931, the disease has not reappeared in sufficient intensity to warrant a resumption of this investigation. This report, admittedly incomplete, is published for assistance to future investigators when, and if, this disease reappears.

The disease was found to be epidemic in the sea herring (*Clupea harengus* L.) throughout the entire Gulf of Maine. The same condition probably existed to a much lesser degree in the alewife (*Pomobolus pseudoharengus* Wilson) of

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that region. Because of the difficulty in obtaining the alewife except during their brief annual migration into fresh water to spawn, work on this host was limited. The common winter flounder (*Pseudopleuronectes americanus* Walbaum) was likewise found to be parasitised in localised areas, where many dead sea herring were available for food.

The causative organism was found to be a pathogenic fungus similar or identical with a fungus reported from both marine and fresh-water fishes of Europe and described as *Ichthyosporidium* (Ichthyophonus) *hoferi*.

II. Historical

The first reference to the effects of a fungus of the type encountered was made by Hofer (1893) who described a serious disease of trout, which he called "Taummelkrankheit" or "whirling disease." This name was derived from the most characteristic symptom, namely, a marked inco-ordination of muscular activity and a resulting general giddiness. Hofer did not attempt to classify the parasite involved, but judging from his description, it was a fungus identical with the one found in trout of the same region by later workers.

Johnstone (1905) referred to a fungus pathogenic to the plaice, *Pleuronectes platessa*, in England. He believed the organism to be of the family Entomophthoracea and near the genus *Conidiobolus*.

Robertson (1908) reported a parasite found in the small flounder, *Pleuronectes flesus*, two years prior to publication. The parasite described corresponds in most details to the herring organism in question. Robertson placed the organism in the genus *Ichthyosporidium* which was created by Caullery and Mesnil (1905) for two species of fish parasites, *I. gasterophilum* and *I. phymogenes*. Robertson included a note in her publication stating that the same type of organism, which had been found in the flounder, was recovered from the body musculature of a haddock sent to her by a fishmonger in Glasgow.

Robertson (1909) described what she believed to be the same organism from the sea trout, *Trutta trutta*, and the description again agrees closely with the present parasite.

Laveran and Pettit (1910) encountered a parasite in the brook and rainbow trout of France, which appears to be a fungus very similar to the organism found in the Gulf of Maine. These authors refused to commit themselves as to the taxonomy of that parasite at the time, and it was not until a year later that the classification was made by the junior author in another publication.

Plehn and Mulsow (1911) next encountered the parasite in trout. They recognised the similarity between it and that described earlier by Hofer. By cultural methods they established its true fungus nature. They believed it to be a Phycomycete resembling the Chytridiales, and created for it the genus *Ichthyophonus* and specifically designated it as *hoferi*.

Following the work of Plehn and Mulsow, Pettit (1913) noticed the similarity between their parasite and the one on which Laveran and he (1910) had worked, and believed that the parasites described by Hofer, Robertson, Laveran

and Pettit, and by Plehn and Mulsow, to be of one and the same genus. Pettit maintained that the name *Ichthyophonus* was a more descriptive name for the parasite, but that it must yield to the priority of the name *Ichthyosporidium*, and, therefore, the name of the fungus should be *Ichthyosporidium hoferi*.

In 1911, Riddell and Alexander encountered the same disease of the flounder, as did Johnstone in 1905. These authors showed, however, that the disease was of bacterial origin and the fungus represented a secondary invader.

Johnstone (1912) reported a fungus in the mackerel, *Scomber scomber*, from the Walney Islands. He believed the parasite to be *Ichthyophonus hoferi* as described by Plehn and Mulsow, and distinct from the fungus he had encountered in the plaice in 1905. The fungus in the plaice as described in 1905 was again encountered by this author in 1919.

In 1914, Neresheimer and Clodi published a comprehensive description of *Ichthyophonus hoferi* including historical summary, morphology, life history and pathogenicity.

Cox (1916) was the first to discover the organism in the Western hemisphere. He found and figured a parasite in the sea herring, *Clupea herangus*, of the Gulf of St Lawrence, unquestionably identical with the organism we encountered in the Gulf of Maine. Cox apparently did not recognise the identity of the parasite, and because of the paucity of material available for study, did not attempt to classify it, other than to state that it was possibly a Myxosporidian.

Léger and Hesse (1923) found a pathogenic fungus, apparently confined exclusively to the intestinal tract of trout. They believed it to be identical with the original *Ichthyosporidium gasterophilum* of Caullery and Mesnil (1905), and reclassified it as *Ichthyophonus intestinalis*.

Léger (1924) described *Ichthyophonus lotae*, from the fresh-water lota. The same author in 1927 and 1929 reported a complete revision of the taxonomic status of *Ichthyophonus hoferi* and other members of the genus as well.

His conclusions, based on cultural results, were that the genus *Ichthyophonus* should be classified as a Zygomycete in the family Entomophthoraceae. This classification agrees with that given by Johnstone (1905) for the parasite of the plaice which he subsequently stated was different from the fungus in the mackerel in 1912. The parasite of the mackerel was similar to *Ichthyophonus hoferi* as described by Plehn and Mulsow. Hence, it is questionable if Léger's reclassification of *Ichthyophonus hoferi* is applicable to the original parasite as described by Plehn and Mulsow.

In 1928, Ellis reported a parasite from the common winter flounder, *Pseudopleuronectes americanus*, in the Passamaquoddy Bay region of the Gulf of Maine. She believed the parasite to be specifically identical with the *Ichthyophonus hoferi* of Plehn and Mulsow. The same author mentions the occurrence of the parasite in flounder taken in the St Andrews, N.B., region of Passamaquoddy Bay in another publication the following year.

In 1929, Léger noted a parasite appearing as a secondary invader in trout

following the occurrence of a neoplastic growth of the stomach wall, the same form of parasite as was described by himself and Hesse in 1923 as *Ichthyophonus intestinalis*.

Daniel (1933, 1933 *a*) published a detailed description of the morphology, life cycle, and pathogenicity of this parasite as it occurred during this investigation in the sea herring. His conclusions are not in entire accord with the subsequent studies made by the United States Bureau of Fisheries and are sufficiently different to justify a more detailed description of the organism.

III. Taxonomy of parasite

This type of parasite was first classified in Caullery and Mesnil's protozoan genus *Ichthyosporidium* by Robertson (1909). In 1911, Plehn and Mulsow discovered its identity as a fungus and reclassified it as *Ichthyophonus hoferi*, n.g., n.sp.

Inasmuch as the two type species of *Ichthyosporidium* described by Caullery and Mesnil (1905), namely, *I. gasterophilum* and *I. phymogenes*, were unquestionably fungi and not protozoa, the genus *Ichthyosporidium*, according to the rules of nomenclature, becomes a valid genus of the fungi and holds priority over the name *Ichthyophonus*. This fact was first advanced by Pettit (1913).

Whether the fungus in question is identical with either of the two type species of the genus *Ichthyosporidium* is doubtful. It is equally doubtful, however, if it is sufficiently dissimilar from the two type species to warrant separate generic classification.

Considerable confusion has also arisen from authors describing new species of the genus *Ichthyophonus*. It is hard to accept some of these new species as belonging to any single genus. Certainly, Robertson (1908, 1909), Johnstone (1912), Cox (1916), Ellis (1928, 1929), Daniel (1933) and probably Laveran and Pettit (1910), Pettit (1913), Plehn and Mulsow (1911), Neresheimer and Clodi (1914) were working with parasites at least generically identical with the one we have encountered. It is extremely doubtful if the parasites described by Johnstone (1905, 1919), Léger (1924, 1929, 1929 *a*) and Léger and Hesse (1923) should be considered as generically related to the parasite in question.

Hence, it is concluded that the parasite which we have found should be classified in the genus *Ichthyosporidium*, as it does not differ sufficiently from the description of that genus by Caullery and Mesnil to warrant separate generic classification. Pending further work, the species is provisionally accepted as *hoferi*, from Plehn and Mulsow (1911).

Although the generic and specific name of this organism may be derived from the literature, its taxonomic status is not so easily determined. The problem is rendered more difficult by the present confusion surrounding the taxonomy of even the higher and better known fungi. Until a better understanding of this fungus, as well as other lower fungi, is reached, it cannot be positively classified.

IV. HOSTS

If the conclusions given above are accepted, the hosts from which parasites of the same genus as that we have encountered in the Gulf of Maine have been described are as follows:

Host	Author	Date	Region
Trout	Hofer	1893	Germany
Flounder	Robertson	1908	England
Haddock			
Trout	Robertson	1909	England
Trout	Laveran and Pettit	1910	France
Trout	Plehn and Mulsow	1911	Germany
Mackerel	Johnstone	1912	England
Trout	Neresheimer and Clodi	1914	Germany
Herring	Cox	1914	Canada
Flounder	Ellis	1928	Canada
Alewife	U.S. Bureau of Fisheries	1931	U.S.A.

V. LIFE CYCLE

The entire life cycle is not completely known for this organism.

The most commonly encountered stage of the parasite in the tissues of the host is the so-called "resting stage." This stage is represented by a spherical, heavy-walled cell which in all probability is slowly growing and preparing for division. All sizes of this resting stage have been observed, from a small, relatively thin-walled uninucleate and binucleate condition to large, thick-walled organisms containing hundreds of nuclei.

From this stage, apparently regardless of the size of the cell, hyphal division may take place. The heavy cell wall gives way and the cytoplasm sends out a coenocytic hypha. This hypha may branch, but seldom branches extensively. It may attain a length of twenty or twenty-five times the diameter of the original spherical organism (Fig. 1). The penetrating abilities of the germinating growth are remarkable, for even connective tissue fails to impede its progress. The mycelium bores through the surrounding tissue and eventually breaks up into a large number of daughter cells, all of which are identical, except for size and quantitative nuclear content, to the parent cells and to the sister cells. The hyphal wall disintegrates and the young spores are so liberated (Fig. I). This life cycle is in accordance with that published by Daniel (1933).

Division by endogenous budding has likewise, but less frequently, been observed. In this method no hyphal growth is produced, and the division of the parent into daughter forms is confined within the cell wall which does not lose its spherical contour (Fig. 2). This method is apparently confined to the larger forms.

Although Daniel (1933) suggested this method of division, his figure illustrating the method is not conclusive, for the organism pictured in his text has not maintained its spherical contour and suggests hyphal development.

The third method described by Daniel, namely "fragmentation," has not been observed in our material. Normal nuclei and cytoplasm have been observed scattered through the tissues, as described by Daniel, but intensive examination has always revealed the presence of the confining hyphal wall, which because of its extreme transparency at times, doubtless, had been overlooked.

It is impossible to predict which method of division any given organism will undergo, and it is equally impossible to determine by which method any given daughter organism was produced.

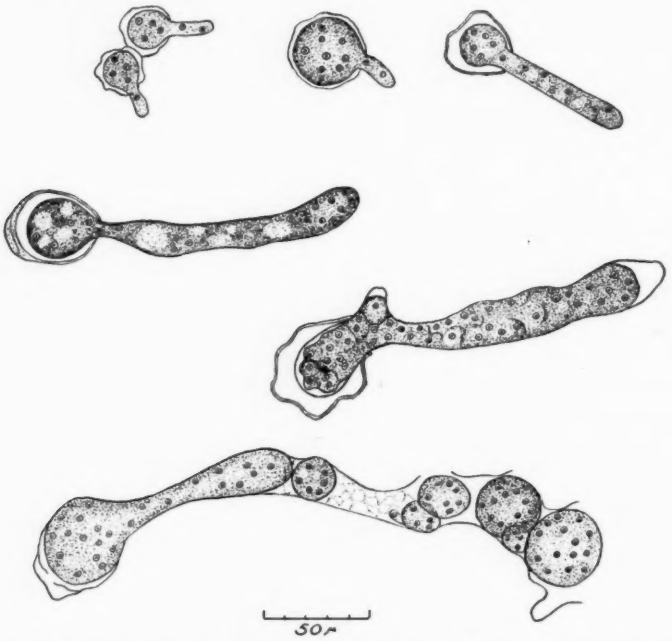

Fig. I. Camera lucida drawings of parasites in various stages of division. Drawings made from stained smears of parasitic cysts. The nuclear count in this figure is not accurate.

The exact method of nuclear division is not known. Pettit (1913) and Caullery and Mesnil (1905) have described a complete mitotic division of the nuclei in the parasites studied. During this investigation, parasites were rarely observed in which practically all the nuclei were undergoing division more or less simultaneously. The nucleus and karyosome elongate approximately three times their normal diameter (Fig. 3). This is apparently followed by a division of the karyosome and subsequent reorganisation into two daughter nuclei. Unfortunately, all the parasites in which nuclear division was observed were stained with eosinmethylene blue. No chromosome complex was observed with this technique, and whether such a division could properly be termed mitotic

is questionable. Nuclear division, in all probability, occurs simultaneously in the same parasite at infrequent intervals, and requires but a short time for completion. Daniel (1933) mentioned nuclear division, although he did not include any detailed description of the process. The uninucleate, heavy-walled, "spherule" type of spore, described by Pettit (1913) and Léger (1929), was not observed. The "spherules" are heavy walled, and supposedly represent the stage in which transmission is effected from host to host. There is no reason to believe that the uninucleate and binucleate forms of the parasite are a necessary part of the life history. From their paucity in heavily infected material it is concluded that these few nucleated organisms are exceptional. The majority of the daughter cells found within the hypha of the mother cell contain at least ten nuclei.

There seems to be no uniformity in the nuclear content of the daughter cells. Apparently the cytoplasm of the mother cell is pinched off in the division without regard to the number of nuclei involved.

VI. Morphology

The so-called "resting stage" of the parasite appears as a spherical cell, fundamentally composed of a heavy wall enclosing the cell proper, or protoplast. Organisms ranging in size from 5μ to 164·5μ have been observed, and within that range there appears to be no difference in internal structure other than in the density of the cytoplasm and the number of enclosed nuclei.

The cell wall of the resting stage is double (Fig. II). The outer wall of a small

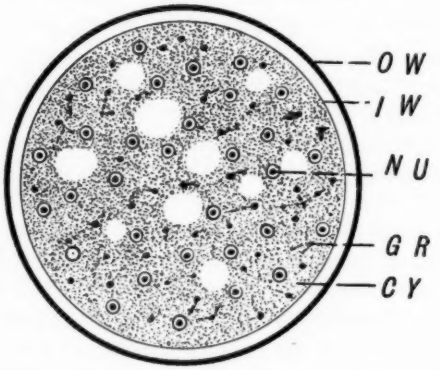

Fig. II. Diagrammatic sketch showing morphology of a "resting stage." *CY* = cytoplasm, *GR* = granules, *IW* = inner wall, *NU* = nuclei, *OW* = outer wall.

organism 7μ in diameter measured 0·2μ in thickness, and a large cell 140μ in diameter had an outer cell wall measuring 2·8μ in thickness. The inner wall is membraneous in nature and extremely difficult to recognise at times.

The cytoplasm in a fresh preparation appears granular to a varying degree of density and often includes clear areas or vacuoles of various sizes.

The nuclei are invisible in the fresh preparation. When stained, they appear to be composed of a thin nuclear membrane embracing a delicate layer of chromatin and enclosing a clear area and a usually central chromatin dot, or karyosome. The normal nucleus is 2·2–2·5μ in diameter, the karyosome being 0·7–9·9μ in diameter. The nuclei appear to be uniform in size, although the position of the karyosome varies.

Rarely, one finds a parasite with unusually large nuclei 4·0–4·4μ in diameter.

The number of nuclei enclosed in a cell varies, but apparently not in direct proportion to the volume of the cell. It is estimated that there may be several hundred nuclei in a single organism 125μ in diameter.

VII. Pathology

A. *Macroscopic*

The herring is the only host of the three attacked by the parasite known to exhibit external evidences of the disease. The growth of the parasite in the subcutaneous and muscular tissues of the herring causes a necrosis of the tissue. A pus sac results under the skin from the accumulation of necrotic tissue. This sac may vary in size from microscopic to well over 3 in. in length. Within the pus sac, parasites in all stages of development may be found. Eventually such a pus sac perforates the "silvery" layer of the intact epidermis, giving rise to the so-called "black spots" which are the most conspicuous external evidence of the disease. These black spots are not accumulations of pigment in the skin, but merely holes which, because they reflect no light, stand out in sharp relief against the bright silvery appearance of the intact skin. When the spots are very small and very abundant, the fish so affected is colloquially known as a "pepper spot." The nature of the larger spots, or holes, is quite apparent as the white subcutaneous tissue may be observed directly through the orifice in the silvery layer of the skin. The skin lesions are believed to be entirely secondary, arising from the spread of the infection from some centre of infection in the internal organs.

The distribution of the internal visible lesions of the herring is almost general. Parasitic cysts are found throughout the internal organs including the heart, liver, spleen, kidneys, intestinal tract, and very rarely on the coverings of the brain and spinal cord. No parasites have been observed in the reproductive organs, but owing to the scarcity of mature fish for study the ovaries and testes were not sufficiently examined to justify any conclusions.

The lesions of the flounder are more confined. No parasites have been observed within the body musculature although the internal organs are infected as severely as are those of the herring. In the alewife, the parasitic invasion has, in all cases, been confined to the heart. The macroscopic internal lesions appear as white, spherical, usually firm, masses or cysts. The viscera of the herring and the flounder may be completely riddled with these cysts

ranging in size from microscopic to that of a small bean. In extreme infections, the heart may be completely overgrown and the entire cardiac cavity completely filled with a dense parasitic mass. The firmness of the cysts varies. They usually appear as discrete masses and at times many of these more or less discrete cysts may be enclosed in a single capsule of host connective tissue. In advanced lesions, there is a tendency for the firm cysts to disintegrate. This liquefaction of cysts is more pronounced in the herring than in the flounder.

B. *Microscopic*

Microscopically, the lesions are essentially similar in all organs and in all hosts. An infiltration of mononuclear cells apparently follows the ingress of a single parasite into the tissues. The tissue immediately surrounding the parasite is replaced by an epitheloid type of tissue, derived apparently from the wandering monocytes. The epitheloid cells are arranged in more or less concentric layers around the parasite and present an appearance not unlike the hard tubercle found in human tuberculosis (Fig. 4). This epitheloid tissue eventually gives way to partial or complete connective tissue replacement, which is believed to represent the chief defence mechanism on the part of the host to prevent further spread of the parasite. In the majority of instances, this mechanism fails to accomplish effectively such a purpose, for the hyphal growth of the parasite directly penetrates the connective tissue barrier and spreads the infection into the surrounding tissue (Fig. 5). As the infection progresses, the parasites increase enormously in numbers and continue their advances, usually being resisted by increasing amounts of epitheloid and connective tissue. The area which once contained a sharply defined "tubercle" becomes a heterogeneous mass of parasites, infiltrating monocytes, epitheloid tissue, connective tissue, and necrotic debris (Fig. 2). Eventually, much of the central tissue dies, and the parasites remain to multiply in a mass of necrotic debris. The area soon becomes large enough to be detected macroscopically as the white cysts described above.

Daniel (1933 *a*) found very little evidence of infiltration in any organ of the herring other than the kidney. However, his description of the microscopic lesions begins with the connective tissue already surrounding the parasite. At this stage in the infection, infiltration is not as marked as in the earlier stages. In the kidney, he found a marked degree of infiltration and described the condition of that organ as that of "acute nephritis." Our studies indicate that infiltration does occur in the kidney but in no greater degree than in any other organ similarly infected. It seems quite probable that this author was influenced by the kidney histology of other animals when determining the degree of infiltration present in the herring kidney. The fish kidney, unlike that of many other animals, normally contains interstitial tissue which apparently serves a hematopoietic function and may easily be confused with a state of infiltration. Although exact conclusions cannot be drawn from Daniel's text

figure of the infiltration found in an uninfected portion of the kidney, it seems quite probable that the tissue illustrated in that figure is normal.

Epidemiology

A. *Incidence of infection*

(1) *Sea herring.* A preserved sea herring taken in 1926 from an unknown part of the Gulf of Maine was found to harbour the parasite. Other fish, taken at the same time, exhibited no trace of the infection, and it is concluded that the disease has existed in the herring of the Gulf of Maine since 1926 and probably had been present, possibly at a subpatent level, prior to that year. From incomplete records kept by the Maine State Department of Agriculture, the disease in the herring was common in 1929, and reached epidemic proportions in the years 1930 and 1931. Although no accurate statistical analyses were kept during the summer of 1932, the Gulf of Maine fishermen report an unusual scarcity of herring, an extremely low incidence of infection, and the existing infection confined, for the most part, to fish over one year in age.

During this investigation, the average incidence of infection in all age groups of the sea herring ran about 70 per cent. throughout the winter and spring of 1931. During the month of July, 1931, the incidence of infection fell, and continued to fall until it had reached an average of 18 per cent. at the time this investigation was terminated. These figures are taken from the records of the Maine State Department of Agriculture.

This decline was first noted in fish of the fingerling size, and occurred simultaneously with the annual migration of offshore fish into shallow waters.

According to Bigelow and Welsh (1924), the eggs of the sea herring are deposited in the fall of the year along the shallower banks and ledges found five to twenty-five miles offshore. The herring fry, when hatched, remain near the site of their birth throughout the following winter. They appear along the Massachusetts shore in the spring, migrating eastward, and arrive along the Maine coast sometime in June or July. The fingerling herring remain in the shallows during the summer, the majority again migrating to the deeper waters for the winter and reappearing in the shoals as yearlings the following spring. The sexually mature herring remain offshore in the deeper waters during most of the year, coming to the shallow banks only at the spawning season.

Apparently the high incidence of infection found during the winter and spring of 1931 was the result of samples taken from a more or less static population living in the shallow water, a region where the transmission of the parasite is most favoured. As the inshore fish were joined by the migrating offshore group the general infection level fell, indicating that this offshore group was less heavily infected, and that the dilution of the infected fish remaining inshore by the migrating offshore group was the primary reason for the decline in the general infection level, and that it was not caused by a seasonal variation of the disease. The assumed lower incidence of infection among the

offshore sea herring is based on the following observations: (1) in the deeper waters, the physical factors are less favourable for the successful transmission of the parasite from host to host, (2) the drop in the incidence of infection among the inshore fish occurred simultaneously with the annual migration from the deeper waters, and (3) the general extremely low incidence of infection among the fingerling fish making their first migration into the shallow waters.

It is concluded that *Ichthyosporidium hoferi* is a natural parasite of the sea herring and reaches epidemic proportions only when certain unknown factors are operating. It is believed that such an epidemic, once initiated, increases in severity, reaches a peak, and subsides to a subpatent level. The unusual scarcity of sea herring in the Gulf of Maine during the past summer indicates that a large number of fish, infected during the past few years, succumbed to the disease.

An item of some interest, although based partly on hearsay evidence, is the apparent cyclic appearance of the disease at intervals of approximately sixteen years. Cox (1916) described a severe epidemic, which was unquestionably caused by the same organism, in the herring of the Gulf of St Lawrence during that year. Cox states that the fishermen of that region told of a similar sickness of the herring in 1898. The reappearance of the disease in 1930 completed the second sixteen-year cycle.

(2) *Alewife.* Little is known of the incidence of infection among the alewife population in the region. Of eighty fish taken during their anadromous migration in the Machias River, ten were found to be infected. At the same time, ninety-three alewives were examined from the Pembroke River and two found infected. It is concluded that the incidence of infection among the alewives of the Gulf of Maine was low, and furthermore, because of the confined lesions, this host plays no part in the natural dispersal of the disease.

(3) *Flounder.* The flounder is believed to represent an accidental host for the parasite. Infected flounder have been taken only in regions where large numbers of dead sea herring were available as food. The endemic areas of infection were extremely localised. This condition is quite understandable when one considers the habits of this fish. The migrations of the flounder are chiefly vertical with the tide.

This fish is a bottom inhabitant in shallow waters, seldom ranging for food. The flounder finds a good source of food, and presumably a good source of this fungus infection, in limited areas where many dead infected sea herring settle to the bottom. It was noted near Eastport, Maine, that the best supply of infected flounder was taken from Lord's Cove, Deer Island, New Brunswick, a small cove where the herring fishermen habitually cleaned the dead fish from their small boats. Of the flounder taken from Lord's Cove 60 per cent. exhibited internal lesions of the disease. In similar coves where the dumping of dead herring was not practised, the percentage of infected flounder ran much lower. The same condition was found to exist near Boothbay Harbour, Maine,

where the only source of naturally infected flounder was in the immediate vicinity of a fish freezing plant. Sea herring were frozen at this plant for use as lobster bait. A small loss of fish overboard accompanied the unloading of the fishing boats, but no such quantities of dead herring were found on the bottom as had been observed at Lord's Cove. The percentage of infection among flounder taken in the immediate vicinity of this fish freezing plant was only ten per cent. Like the alewife, the flounder probably plays no part in the natural spread of the disease.

B. *The mechanics of infection*

Infection of fresh sea herring hosts is probably accomplished by the ingestion of resting stages of the parasite, liberated from the ruptured pus pockets of infected fish. The alewife is probably likewise infected during its occasional contact with the herring in deep waters. The flounder, being a carnivorous fish, and not a plankton feeder, is probably infected by the eating of infected herring.

These conclusions are not indisputable because of the general lack of convincing experimental evidence.

It is possible to establish experimental infections by feeding infected material to fresh hosts. Successful cross-infection experiments were established from the herring to the flounder. Because of the inadaptability of the herring to laboratory conditions and the uncertainty of obtaining uninfected experimental animals, the infections could not be reversed. It was possible to obtain flounders with a reasonable degree of certainty as to their freedom from the disease in regions where a large number of these fish had been examined and no trace of the parasite found. The flounder is capable of withstanding laboratory conditions in any region where running sea water is available and makes a satisfactory laboratory animal. Flounders were seined from the Sheepscott River near Spectacle Island. During the summer, a total of 230 flounders from this general vicinity were examined for infection and none found to be positive. Fish from six to ten inches in length were used as experimental animals and maintained in the regular fish hatching troughs at the Boothbay Harbour hatchery. Controls were picked at random from the captured fish and kept in separate troughs, but in the same series of troughs, as were the experimental fish. The experimental fish were starved for a period of one week. At the end of this time they were forcibly fed a suspension in sea water of parasitic cysts taken from heavily infected herring. The infections were given *per os* by means of a soft rubber catheter and a 20 c.c. Luer syringe. The suspension of parasites was not standardised, but approximately 7·5 c.c. of the suspension was given each time. Considerable difficulty was experienced by regurgitation on the part of the flounder, and although repeated inoculations were administered it is doubtful, in some cases, if the fish received many parasites. It is believed that the rejection of the inoculum by the fish accounted, to some degree, for the low percentage of positive infections found when the fish were autopsied. Two series

of cross infections were attempted. The experimental flounders were kept alive as long as circumstances permitted. Infections were considered positive only when microscopical examination of the viscera revealed the presence of parasites. In the first series thirty-seven fish were used, twenty-five as experimental animals and twelve as controls. Six of the experimental animals were found positive at autopsy. In the second series of infections, fourteen fish were used, nine as experimental animals and five as controls. One of the experimental hosts in the second series was positive at autopsy. The controls in each series were uniformly negative.

These limited experiments indicate that some form of the parasite found within the cysts of the infected herring is capable of transmitting the infection. Prolonged searches of the stages encountered within the cysts were indicative of the heavy-walled resting stage being the most likely stage to accomplish the transmission. Daniel reports the recovery of the parasite from the faeces of gulls which had been feeding on infected herring. The parasites so recovered appeared to be alive as they appeared as normal as those taken directly from the fish. If this resting stage is capable of withstanding the unfavourable environment found in the digestive tract of the gull, it is probable that it is able to withstand, for a limited time, the environment found in sea water.

Experimental evidence is totally lacking as to the actual mode of penetration of the parasite into the tissues of the fish. From the behaviour of the resting stage within the tissues, it is not improbable that the production of hyphae would be a possible and effective means of penetration. The normal stasis of the intestinal contents of the fish, particularly in the pyloric caeca, would afford a suitable opportunity for penetration in this fashion.

From the widespread occurrence of the lesions throughout the body of the host, it is concluded that the distribution of the parasite, once penetration is effected, is accomplished by way of the blood stream or the lymphatics. The migrating stage of the parasite is unknown. No parasites have been observed actually within a blood vessel, and it is not impossible that an unknown stage in the life history accomplishes this purpose.

C. *The mechanics of the varying degree of infection among susceptible hosts*

The mechanics of the varying degree of infection among the three susceptible hosts by *Ichthyosporidium hoferi* is probably best explained by the difference of feeding habits and distribution of the individual hosts. The sea herring, being plankton feeders, swim with their mouth open, straining the food organisms from the water as they go. Their close approximation in schools gives the parasite a favourable opportunity to be immediately consumed by a fresh host when liberated from an infected host. Once the infection becomes established in a school of sea herring, the members are exposed to constant reinfection. The frequent mixing of the schools and the great extent of their migrations aid in the general spread of an infection in this host.

The alewife, remaining in deep water except for very limited runs into fresh water for spawning, probably finds considerably less contact with the parasite than does the herring. The natural factor of dilution in the deeper waters would play a much larger rôle in opposing the successful transmission of the parasite than in the shallows. Hence, the alewife probably seldom encounters the parasite except during its annual migration through the shallows to spawn, and during its infrequent association with the herring.

The flounder, as previously explained, is exposed to infection only as it utilises dead infected herring as food. It is not, therefore, exposed to a constant infection as is the herring, but more probably accidentally incurs an occasional massive inoculation as it happens to feed in a very restricted area.

The reason for the apparent immunity of other poikilothermic animals of the Gulf of Maine to this disease is obscure. Some search was made for the organism in 2 brown trout, 38 harbour pollock, 21 codfish, 8 sculpin, 144 smelt, 3 hake, 10 mackerel, and 10 squid taken from various parts of the Gulf of Maine, but without success. This may be on account of an insufficient number of hosts being examined, unsuitability of the hosts for the growth of the parasite, or to the lack of contact with the parasite. The first two reasons seem more probable, as practically all of the animals mentioned feed at one time or another on the sea herring.

IX. Culture of parasites

Ichthyosporidium hoferi taken from infected herring was successfully cultivated on four types of agar slants. The media used were (1) Henrici's routine medium for fungi, (2) a modification of (1) enriched with fish broth, (3) Sabouraud's medium for fungi, (4) fish glycerine agar. The fungus is apparently a facultative aerobe, growing at 20° C. The growth is very slow, requiring about two weeks to show any evidence of germination. The growth on the slants never became profuse, but successful transfers were made. There was little or no evidence that the organism grew differently in culture than in the tissues of the host.

X. Summary and conclusions

1. A fungus disease of epidemic proportions was found in the common sea herring (*Clupea harengus*) throughout the Gulf of Maine.

2. The fungus was also found to infect the common winter flounder (*Pseudopleuronectes americanus*) and the alewife (*Pomobolus pseudoharengus*).

3. The causative agent was found to be a species of fungus belonging to the genus *Ichthyosporidium* Caullery and Mesnil (1905). The specific name is tentatively accepted as *hoferi* Plehn and Muslow (1911).

4. The organism is believed to be a normal parasite to the herring and reaches epidemic proportions only when certain unknown factors are operative.

5. The causative organism was found in herring preserved in 1926, and it is believed that the epidemic has been increasing in severity since that time.

6. It is believed that such an epidemic, once initiated, increases in severity, reaches a peak, and subsides to a subpatent level. The peak is believed to have been reached in 1931.

7. The life history and effects of the organism in the herring and flounder are described.

8. The herring is believed to acquire the infection by the ingestion of parasites liberated from fish in the same school.

9. The flounder is believed to acquire the infection by the consumption of infected herring.

10. The alewife is believed to acquire the infection by ingestion of the parasite during its infrequent association with the herring.

11. Infection is believed to be established by way of the alimentary canal and, once established, to spread throughout the host by way of the blood stream or the lymphatics.

12. Direct cross infection from the herring to the flounder establishes the theory that the parasites in these two hosts are one and the same organism.

13. Direct cross infection experiments from the herring to the flounder eliminate the necessity of an intermediate host.

14. There is no reason to believe that this parasite is capable of infecting warm-blooded animals.

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EXPLANATION OF PLATE I

Fig. 1. Cellular division of parasite. The cytoplasm of the parasite has completely divided into a large number of daughter parasites. The hyphal wall of the mother parasite has not ruptured. Smear of parasitic cyst. × 78.

Fig. 2. Cellular division of parasite within advanced lesion. The double-walled "resting stage," hyphal development, and endogenous budding are found within this lesion. The parasites are apparently rapidly multiplying, the epithelioid cells formerly surrounding them are dead and disintegrating, and there is a heavy encapsulation of connective tissue present. Section of flounder heart. × 360.

Fig. 3. Nuclear division of parasite. *D*, dividing nucleus. *N*, normal nucleus. Section of flounder liver. × 780.

Fig. 4. Detail of early lesion. Showing the host's first response to the parasitic invasion of an uninfected area. Note the centrally located parasite, the surrounding epithelioid tissue which has replaced the normal tissue, this being seen in the extreme periphery of the "tubercle." Section of flounder spleen. × 360.

Fig. 5. The spread of the parasite into uninfected tissue. A more advanced lesion than that shown in Fig. 2. The central tissue of the cyst is completely liquefied and the parasites, by hyphal development, are penetrating the protective connective tissue capsule. Section of flounder spleen. × 78.

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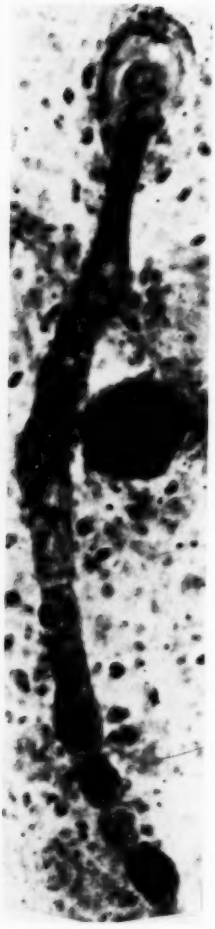

Fig. 1

Fig. 2

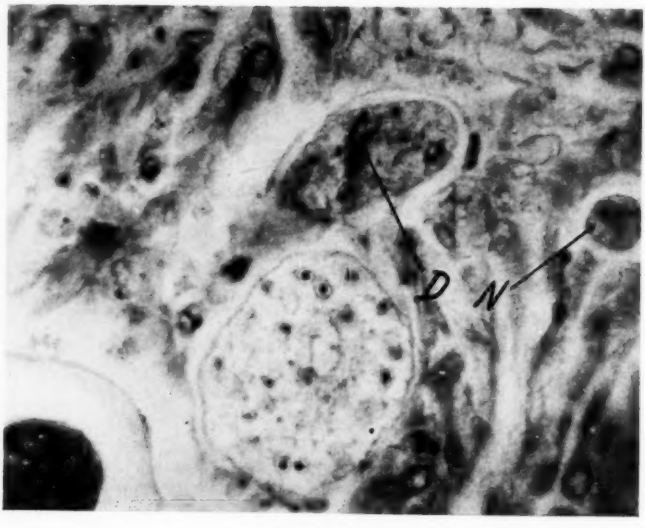

Fig. 3

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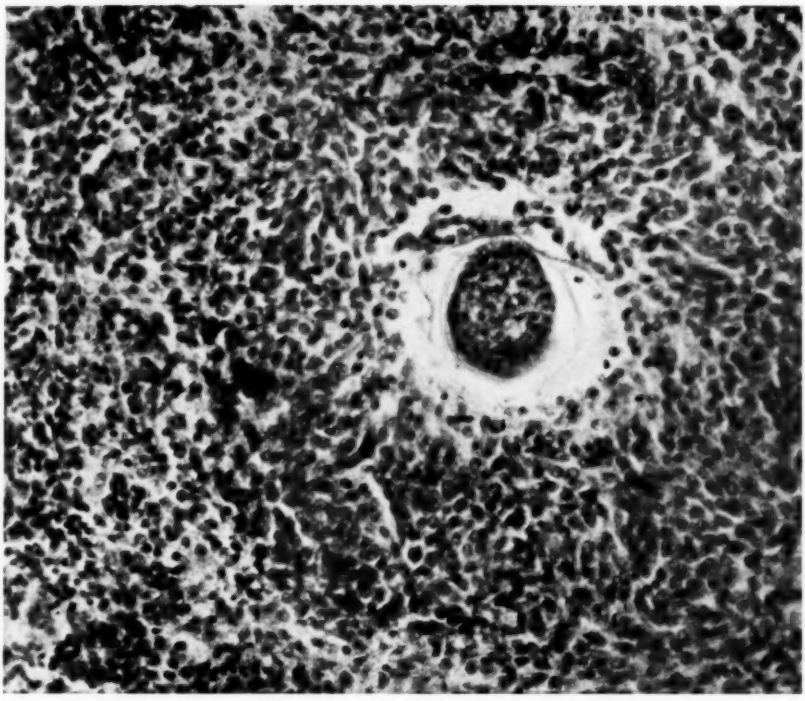

Fig. 4

Fig. 5

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OBSERVATIONS UPON *EMBADOMONAS INTESTINALIS* IN CULTURE

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(With Plate II)

I. Nomenclature

The nomenclature of the genus *Embadomonas* Mackinnon, 1915 has been reviewed by Wenyon (1926), and to refer to the matter here, would be unnecessary but for a recent paper by Wenrich (1932) in which he states that the generic name *Embadomonas* is a synonym of *Retortamonas* Grassi, 1879. He asserts that the name *Embadomonas* is no longer valid and that the genus of flagellates which has been known by it should henceforth be called *Retortamonas*. I have recently (Bishop, 1932) discussed the papers by Grassi (1879, 1881, 1882 and 1883) which deal with these flagellates, and since reading Dr Wenrich's paper I have referred to them again. The matter is complicated by Grassi, in his later papers (1881, 1882 and 1883), changing the generic name of *Retortamonas* Grassi, 1879 to *Plagiomonas* Grassi, 1881. No explanation is given of this change. It is extremely difficult for me, as I stated previously (1932), to make a decision upon the exact systematic position of flagellates described and illustrated as are some in these papers, and I cannot agree that Grassi's description and two figures of *Plagiomonas* (=*Retortamonas*) *gryllotalpae* sufficiently resemble *Embadomonas* to justify a change in this generic name. In a later paper Grassi (1888) defines *Plagiomonas* as having "Due flagelli anteriori, diretti anteriormente ed uno posteriore (caudale)." Dr Wenrich makes no reference to this paper. This definition of the genus, by its discoverer himself, appears to me to be of great importance, as it differs in the number of flagella from the earlier descriptions. If *Plagiomonas* is thus later defined as having three flagella then it cannot be a synonym of *Embadomonas* which has only two. This definition, therefore, supports my opinion that a change in generic name of *Embadomonas* on the evidence in Grassi's papers is unwarranted. In the following description of the species found in man, and first described by Wenyon and O'Connor (1917) as *Waskia intestinalis* (=*Embadomonas intestinalis* Chalmers and Pekkola, 1918), the generic name *Embadomonas*, instituted by Mackinnon (1915), is therefore still used.

A list of species of *Embadomonas* and their hosts has been given previously (Bishop, 1931), and further additions are found in Wenrich's paper.

II. Material

The strain of *Embadomonas* described below was isolated, on 26. i. 32, from the stool of a patient, by Mr Clifford Dobell, F.R.S. Two cultures (23rd and 34th subcultures) were posted by him, on 24. v. 32, to Dr Toynbee Wight in California, who recovered the flagellate from one and cultivated it for some time. Dr Toynbee Wight posted back a subculture to Mr Dobell, which he recovered in culture. He called this strain, which had twice crossed the Atlantic, the G.W. strain (1st subculture). Dobell mentions it in the *Report of the Medical Research Council* (1933). He very kindly gave me a tube containing the 17th serial subculture of this strain and its descendants provided the material for the present study. My initial culture (G.W. 17) was growing upon a medium consisting of an inspissated horse-serum slope covered with Ringer egg-white. It has grown very thickly upon this medium and still produces excellent cultures and rich crops of cysts; but it also grows well upon an inspissated horse-serum slope covered with inactivated horse-serum diluted 1 : 10 in 0·5 NaCl solution, and encysts well in this medium It will not grow in the latter liquid medium alone.

Mr Dobell had been cultivating *E. intestinalis* at 37° C. but found that it could survive for relatively long periods at room temperature. These findings were confirmed by my own results. Cultures grown at 37° C. lived for 5–7 days, but if put on the bench (17–20° C.) on the 3rd or 4th day, at which time they were richest, healthy embadomonads were present for 28–52 days. Subcultures from cultures grown at 37° C. were kept at 23–24° C. and excellent growths resulted, the cultures lasting for at least 10–14 days. Subcultures made from cultures grown at 37° C. and kept at 17–20° C. produced rich growths, but did not reach their maximum for at least 14 days, as compared with the 3rd or 4th day when grown at 37° C. After a month or 5 weeks these cultures grown at room temperature were still rich in flagellates in addition to innumerable cysts. Strains have been grown continuously at 17–20° C. for 4 months and are quite as rich as those grown at 37° C. Attempts were made to grow them at 11° C., but though the flagellates remained alive and appeared healthy, no multiplication could be observed. At 4° C. the flagellates begin to die in a few days.

Hogue (1921) first cultivated *Waskia* (=*Embadomonas*) *intestinalis* at 37° C. in a medium consisting of egg-white cooked in 0·7 NaCl solution, but the flagellates were transferred every other day. Wenyon (1921) cultivated *E. intestinalis* and also embadomonads from the guinea-pig, rat, tortoise and frog in Hogue's medium, and also in a soft rabbit-blood-agar medium, the flagellates from both warm and cold-blooded hosts growing at 24–30° C. Faust (1923) states that *E. sinensis* (from Chinese patients) grew more rapidly at 30° C. than at body temperature, but he does not mention in what medium. Collier and Boeck (1926) found that *E. cuniculi* grew both at 37° C. and at room temperature. When grown at 37° C. the cultures were viable for only 10 days,

whereas those grown at room temperature lived 35–40 and in one case for 60 days. They obtained a growth of the flagellates in a medium consisting of egg slants covered with sterile hay infusion but growth was uncertain, and of short duration. They concluded that *E. cuniculi* is not coprozoic, but a "non-pathenogenic obligative parasite."

Attempts have been made to cultivate *E. intestinalis* at 37 and 17–20° C., in sterile hay infusion, and also in dilutions in tap water of human faeces from a person not infected with intestinal Protozoa, but they have been invariably unsuccessful. Whilst working previously with the intestinal flagellates of frogs and toads dilutions were made with tap water of faeces of these Amphibia infected with *E. dobelli*, but the flagellates never multiplied, and could not be found after 48 hours. Plate cultures of horse-serum Ringer egg-white were made from intestinal contents of frogs and toads some of which were infected with *E. dobelli*, but growths of this flagellate were never obtained, though the conditions were found to favour the growth of coprozoic forms. From the existing evidence, therefore, it would appear that, though some species of *Embadomonas* are known to live over a wider range of temperature than is usual for intestinal Protozoa, they need the culture conditions requisite for true Entozoa and cannot be regarded as coprozoic.

Embadomonas intestinalis and its cysts are difficult to fix and stain well, and the best results have been obtained by fixing in aqueous Bouin's solution and staining with aqueous iron haematoxylin.

III. Infection experiments with *Embadomonas intestinalis* and tadpoles of *Bufo vulgaris*

That *Embadomonas intestinalis* grows well at room temperature suggests that it may live in the alimentary canal of some cold-blooded host as well as in man. As will be shown later it differs from *E. dobelli* of frogs and toads in the presence of a karyosome, and slightly in size, but the cysts are strikingly similar in structure. As a few tadpoles of *Bufo vulgaris*, reared free from Protozoa, were available it was decided to test if it were possible to infect them with *E. intestinalis*. These tadpoles were kept in sterilised tap water, and fed upon boiled dog biscuit and faeces from a human being who was negative for Protozoa as proved by repeated examination. Cultures of *E. intestinalis*, grown at 17–20° C. and rich in cysts, were sedimented in Ringer solution to wash away the serum, which is harmful to the tadpoles, and the concentrated cysts and flagellates were mixed with faeces and fed to the tadpoles. This mixture they ate voraciously. Similar methods have been used (Bishop, 1934) successfully in infecting tadpoles with strains of *Trichomonas*.

On 19. v. 33 twelve tadpoles were fed with concentrated cysts and flagellates of *E. intestinalis*, and twelve were used as controls. Two days later a tadpole was killed and dissected and its gut examined, but no flagellates were found. A second tadpole was killed and examined 2 days later with the same negative result. Eight days later two more tadpoles were killed and examined

and both were negative. The remaining tadpoles were fed again with a large quantity of cysts and flagellates. Two tadpoles killed and examined 2 days later were both negative. The remaining six tadpoles were fed again but on subsequent examination all were negative. The controls were negative for Protozoa when examined.

The small number of experiments (due to the few tadpoles free from Protozoa available) makes a definite statement impossible; but as not one of the twelve tadpoles fed with rich crops of *E. intestinalis* became infected it seems very improbable that tadpoles of *Bufo vulgaris* are hosts to this flagellate. If *E. intestinalis* does live in a cold-blooded host as well as in man, as the wide range of temperature at which it can be cultivated suggests, then we must look farther for this second host.

IV. Morphology and method of division of *Embadomonas intestinalis*

Embadomonas intestinalis has been described by Wenyon and O'Connor (1917) who first discovered it, Dobell and O'Connor (1921), Broughton Alcock and Thomson (1922), Jepps (1923) and others. These workers agree very closely on the question of the size of the flagellate, the lengths ranging between 3·5 and 9μ, the majority lying between 5 and 6μ. The living flagellates of the G.W. strain when grown at 37° C. measured 6–10μ in length, the majority being 7·5–9μ; but on fixation, however carefully conducted, shrinkage occurs, and the length of the majority was 5–7μ. When grown at 17–20° C. the flagellates were noticeably larger, measuring 9·5–14μ when alive, the majority being 10–12μ; but on fixation the length of the greater number was 6–9μ. With continual cultivation at 17–20° C. the size did not increase further and *E. intestinalis* never became as large as *E. dobelli* whose length, when fixed and stained, was 6–14μ, the majority measuring 9–11μ. *E. dobelli* is broader than *E. intestinalis* and appears more robust.

In the living flagellate the two flagella can be studied. There is a long anterior one, serving for propulsion, and a thicker, shorter one lying in the well-defined cytostome and projecting well beyond it. The continual flickering movement of this cytostomial flagellum is characteristic of the flagellate as is also the jerky movement of the body. The cytoplasm usually contains many food inclusions, the flagellate feeding upon the bacteria present in the culture. The edge of the cytostome is supported by a delicate fibril, or pair of fibrils as in *Chilomastix*; it is difficult to be certain which in so small a creature.

In the living flagellate the spherical nucleus can be distinguished as a denser area at the anterior end of the body. When fixed and stained (Figs. 1, 2 and 3) it appears as a deeply staining central karyosome, usually spherical, surrounded by a clear area limited by the spherical nuclear membrane. On the inner side of this nuclear membrane is a thin layer of chromatin. In optical section, therefore, the nucleus appears as a fine ring with a granule in the

centre. The two flagella arise from a pair of small siderophilic basal granules which lie close together on the surface of the nuclear membrane at the anterior end of the cytostome.

In cultures grown at all three ranges of temperature (37, 23–24 and 17–20° C.) individuals (Fig. 4) having two nuclei, two sets of flagella and basal granules, and two cytostomes, which usually lie on opposite sides of the body, were found, as in *E. dobelli* (Bishop, 1931), but they were not very numerous. References to observations by other workers on such double individuals in *Embadomonas* have been given in the paper cited above.

Apart from a few isolated observations which do not appear to form part of the normal process (*vide infra*) no account of the method of division of *E. intestinalis* has been published. The division of the body of *E. intestinalis* can be studied in the living flagellate, but the nuclear division can only be investigated in fixed and stained material. The earliest stages found were flagellates having three or four flagella (Figs. 5 and 6) which arise close together, above the anterior end of the cytostome, from a group of basal granules. These lay so close together that it was impossible to count them. It has not been possible to find early stages in the outgrowth of these new flagella. At this stage the karyosome appears to grow larger and sometimes (Fig. 5) to be breaking up into several granules. The living flagellate becomes rounded, the cytostome disappears and the four flagella separate into two pairs and move apart. The cytostomial flagellum loses its characteristic movement and is difficult to distinguish from the others. In some stained preparations (Fig. 7) each individual flagellum arises from a separate basal granule. A darkly staining thread, or centrodesmose, joins one granule of each opposite pair. One granule of each pair, therefore, functions as a centrosome as well as a basal granule, as in *E. dobelli* (Bishop, 1931). The centrodesmose lengthens as the pairs of flagella and their basal granules move farther apart. As the karyosome increases in size the nuclear membrane loses its layer of chromatin and becomes less well defined. It becomes spindle-shaped, with a pair of flagella and basal granules at either pole (Figs. 8 and 9), but no spindle fibres were ever seen. The karyosome breaks up into discrete granules of which there appear to be five or seven, but it has been impossible to count them exactly. That these granules divide is to be concluded from specimens (Fig. 10) in which the nucleus contains a larger number of smaller granules segregated towards the poles, but no direct evidence of their division has been found. These granules come together in two dense masses at the poles (Fig. 11) of the spindle-shaped nucleus. The body of the flagellate begins to elongate (Fig. 12), and new cytostomes form at either end. The characteristic appearance of the two cytostomial flagella becomes evident. The elongated centrodesmose still unites one basal granule of each opposite pair. The daughter nuclei begin to re-form, at first with large central karyosomes and without peripheral chromatin, but later (Fig. 13) the normal nuclear structure appears. The body of the flagellate constricts across the equator. The daughter individuals appear to attempt to swim in opposite directions so that

the middle of the body is pulled out into a fine connecting strand which, in stained preparations (Fig. 14), seems to be formed wholly of the centrodesmose. This finally breaks and the two daughters swim away. It has not been possible to discover how soon after division of the body absorption of the centrodesmose occurs. No difference, except in size of body, is found in the division of individuals grown at 17–20° C. from those grown at 37° C.

Apart from its smaller size, *E. intestinalis* differs from *E. dobelli* in possessing a karyosome, a structure not usually present in the resting nucleus of the species from frogs and toads. The main outline of the division process is similar in the two species, but, in spite of its smaller size, the granules in the dividing nucleus of *E. intestinalis* are much more sharply defined than in *E. dobelli*.

In the nucleus of *E. cuniculi*, Collier and Boeck (1926) describe a central spherical or oval karyosome which is "connected by an intranuclear rhizoplast to the centrosome lying on the nuclear membrane." This centrosome, they assert, is connected by an "extranuclear rhizoplast" to the two blepharoplasts from which the flagella arise. Such rhizoplasts have not been seen in *E. intestinalis*. In the division of *E. cuniculi* these authors describe the formation from the karyosome of a spireme, which gives rise to two bivalent chromosomes. They also describe a "very delicate intranuclear spindle, with a centrosome at each pole" and a paradesmose (=centrodesmose) connecting the centrosomes. Further, a transverse division of the rod-like chromosomes is described. A spindle with fibres, such as figured by Collier and Boeck (Plate 15, figs. 6, 7 and 8), was never seen in *E. intestinalis*. Whereas in *E. intestinalis* and in *E. dobelli* (Bishop, 1931) the cytostome is absorbed early in division and daughter cytostomes are formed in the developing daughters before their separation, Collier and Boeck figure the original cytostome as persisting all through division. They give no account of the origin of the new flagella and their basal granules, but (Plate 15, fig. 8) show an individual, with a spindle-shaped nucleus, provided with two fully formed cytostomes (presumably the original one and a new but completely developed one) and two sets of flagella and their granules. Such a stage leads on to individuals with two nuclei, two cytostomes, and two sets of basal granules (Plate 15, fig. 11) which correspond to double individuals in *E. intestinalis* and *E. dobelli*. Wenyon and O'Connor believe such forms in *E. intestinalis* to be a stage in division, which is also the interpretation given by Jepps and by Broughton Alcock and Thomson. Geiman (1932) gives a few stages in the division of *Retortamonas* (=*Embadomonas*) *caudacus* from the larvae of Gyrinidae. His interpretation is rather difficult to follow, but from the few isolated cases he studied he appears to conclude that division of the nucleus takes place at the anterior end of normally shaped individuals, thus giving rise to individuals with two nuclei and two cytostomes at the anterior end. The body, according to him, subsequently elongates and the nuclei and cytostomes appear to migrate to opposite ends.

That a stage with two anterior nuclei and two cytostomes is not normal in the division of *E. intestinalis* and *E. dobelli* I am quite certain, but how such double

individuals arise I have been unable to discover. Long observation upon them failed to reveal division of the body, nor did Collier and Boeck observe it in *E. cuniculi*. I can only suspect that in some individuals the nuclei migrate towards one another after the division of the nucleus and the formation of the cytostomes is complete.

V. THE CYST OF *EMBADOMONAS INTESTINALIS*

The cyst of *Embadomonas intestinalis* is a minute, opaque, pear-shaped or oval structure. The average length of the living cyst, when grown at 37° C., is 5–7μ, but a slight shrinkage occurs on fixation, and such cysts measure 4–6μ. The living cysts from cultures grown at 17–20° C. measure 6–7μ but are slightly smaller on fixation. Two refractive lines can be seen converging towards the apex of the living cyst but no other internal structure is visible. With iodine the cysts stain faintly and diffusely. When fixed and stained they resemble the cysts of *E. dobelli* very strikingly in their internal structure. Cysts have been studied from cultures grown at 37 and 17–20° C. and no difference has been found in their structure. The structures in the cysts seem to be variable and are difficult to interpret. Cysts from cultures in which encystment was only just beginning were oval or pear-shaped (Figs. 16–17). The nucleus lay in the centre of such cysts. In some specimens (Fig. 16) the nuclear membrane, though discernible, was without chromatin, and a large karyosome lay in the centre. The basal granules were distinct and lay close to the nuclear membrane. A few delicate fibrils and one thicker one were sometimes seen and probably represented the flagella and cytostomial fibril. In other cysts (Fig. 17) a thick layer of chromatin was seen on the inner side of the nuclear membrane and the karyosome was smaller than in the previous type of cyst. In almost all specimens the nucleus appeared larger in proportion to the cyst than it did in the flagellate stage. In older cysts the two basal granules lay at the broader end (Figs. 15, 18 and 19). One, or sometimes two, granules lay in the centre of the cyst and probably represent the karyosome. Two siderophilic lines converging towards the apex of the cyst were usually present, and represent, I believe, the elongated nuclear membrane and its accompanying layer of chromatin. Other, less well-defined, siderophilic fibrils probably represent the cytostomial fibril and the flagella. A number of small, deeply staining granules were sometimes present, scattered throughout the cytoplasm, but there was no indication of how they arose. Many cysts have been studied without finding any evidence of division occurring in them, or of the encystment of double individuals.

References to descriptions of cysts in different species of *Embadomonas* have been given and discussed previously (Bishop, 1931), so that it will be necessary here to mention only those dealing with *E. intestinalis*. Wenyon and O'Connor interpret the long siderophilic lines as the elongated nuclear membrane. They state that the karyosome tends to become dumbbell-shaped or to be divided into several parts. Dobell and O'Connor describe a specimen of cyst

which is not typical of *E. intestinalis*, though somewhat similar forms have occasionally been found in crops of cysts from the G.W. strain. Broughton Alcock and Thomson interpret the elongated siderophilic line as the cytostome and the central granules as part of the nuclear structure, the nuclear membrane being in most cases invisible. Wenyon later (1926) suggests that the line represents the margins of the cytostome and not the nuclear membrane. But from observations on many cysts of *E. intestinalis*, *E. dobelli* and *E. blattae* I still hold the opinion that these long lines represent an elongated siderophilic nuclear membrane.

The amount of siderophilic matter in the cyst compared with the free flagellate is very striking.

VI. Summary

1. *Embadomonas intestinalis* has been cultivated on an inspissated horse-serum slope covered with Ringer egg-white or with inactivated horse-serum diluted 1 : 10 in 0·5 NaCl solution, at 17–20° C. as well as at 37° C.

2. Attempts to infect tadpoles of *Bufo vulgaris*, bred free from Protozoa, with *E. intestinalis* have failed.

3. In the division of *E. intestinalis* there is duplication of the basal granules and flagella with the formation of a centrodesmose between one granule of each pair. The cytostome disappears and new ones are formed when nuclear division is complete. No spindle fibres are formed but the nucleus becomes spindle-shaped and discrete chromatin granules are present.

4. Double individuals occur but they do not represent a stage in normal division. Long observation failed to reveal division of such individuals.

5. Cysts of *E. intestinalis* have been found in cultures at all temperatures studied. The nuclear membrane and peripheral chromatin is elongated and stains intensely, and the two basal granules are prominent. A siderophilic mass in the centre probably represents the karyosome. There is no evidence that division occurs in the cyst.

I wish to record my thanks to Mr Clifford Dobell, F.R.S., for the gift of the strain of *E. intestinalis*, and also for the stimulating interest which he has shown in this study of it.

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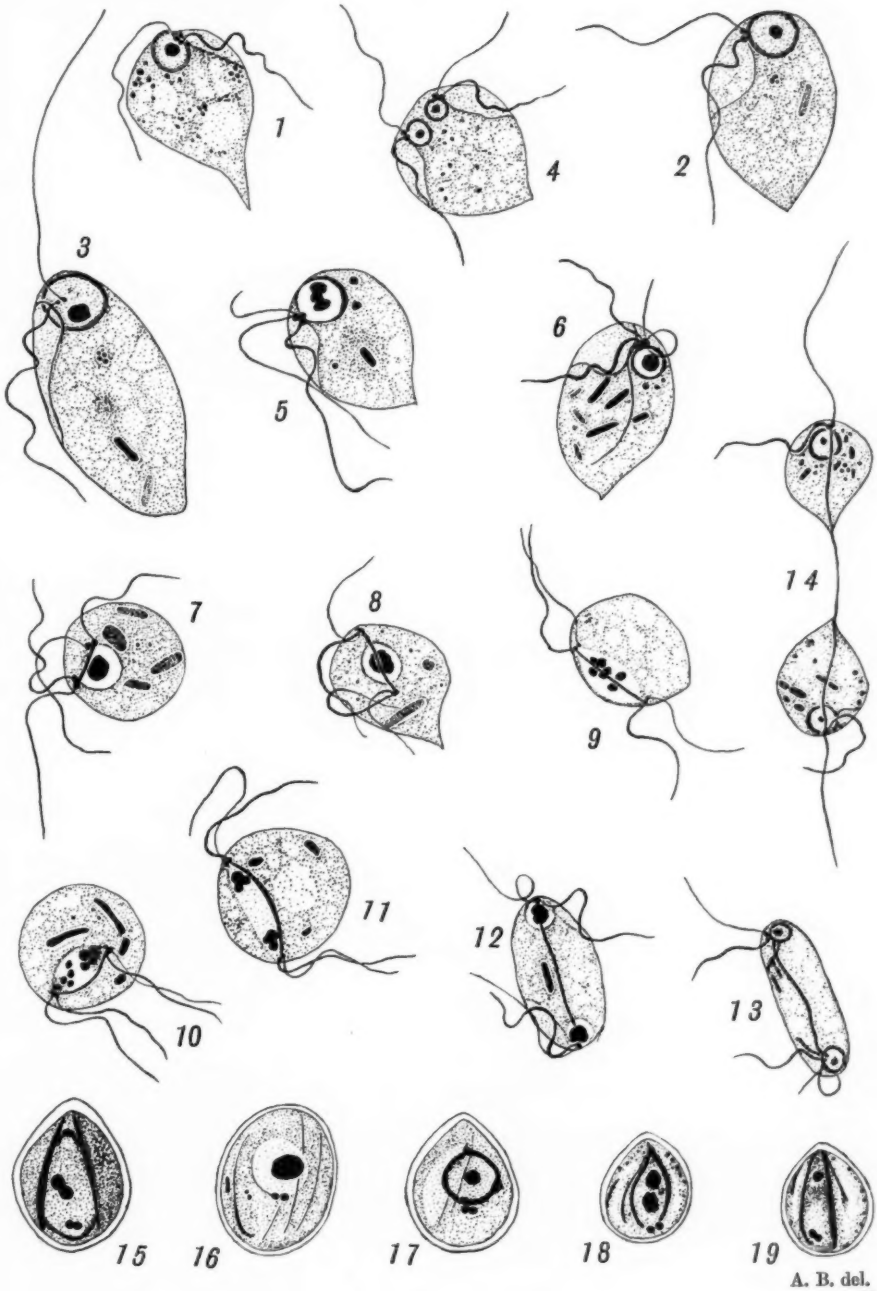

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EXPLANATION OF PLATE II

Embadomonas intestinalis

Fig. 1. Individual grown at 37° C. showing nucleus, flagella, and basal granules.

Figs. 2, 3. Same grown at 17–20° C.

Fig. 4. Double individual grown at 37° C.

Fig. 5. Early stage of division showing three flagella and basal granules. Grown at 37° C.

Fig. 6. Same with four flagella. 37° C.

Fig. 7. Flagella and basal granules drawing apart in pairs. 37° C.

Fig. 8. Further advanced stage than Fig. 7. 37° C.

Fig. 9. Spindle-shaped nucleus. Karyosome broken up into granules. 37° C.

Fig. 10. Larger number of granules moving towards the poles of the spindle-shaped nucleus. 37° C.

Fig. 11. Chromatin forming in masses at the poles of the nucleus. 37° C.

Fig. 12. Reorganisation of daughter nuclei and cytostomes. 37° C.

Fig. 13. More advanced stage than Fig. 12. 37° C.

Fig. 14. Daughter flagellates separating. 37° C.

Fig. 15. Cyst. 17–20° C.

Figs. 16, 17. Newly formed cysts showing enlarged karyosome and the original shape of the nucleus. 17–20° C.

Figs. 18, 19. Mature cysts. 37° C.

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THE EXPERIMENTAL INFECTION OF AMPHIBIA WITH CULTURES OF *TRICHOMONAS*[1]

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I. Introduction

The natural method of transmission, from host to host, of intestinal flagellates belonging to the genus *Trichomonas* is of interest, as no authentic encysted stage is known for the majority of species. In the past cysts have been described, by different workers, for several species of *Trichomonas* (Bishop, 1931 *a*), but most of these descriptions have now been proved incorrect. During the past three and a half years I have been studying and cultivating the intestinal Protozoa of frogs and toads, and I have been able to make a detailed study of *Trichomonas batrachorum*. The faeces of many frogs (*Rana temporaria*) and toads (*Bufo vulgaris*), some very heavily infected with *T. batrachorum*, have been examined without finding cysts which could be assigned to this species. Cultures have been made from many of these animals. Twenty-six strains have been maintained for periods varying from fourteen days to nine weeks through different numbers of subcultures; one strain from the frog has been cultivated for five months and two others for three months, whilst one strain from the toad has been cultivated for thirteen and a half months and another for five months. These strains have been examined microscopically at frequent intervals, sometimes daily, and at all ages of the cultures, and many preparations have been made and studied without finding any cysts.

Descriptions, by several workers, of encystment of *Trichomonas hominis* have not been confirmed, and no true cyst is known for this species nor for the trichomonads from the intestine of macaque monkeys. A series of experiments was performed (Bishop, 1930) upon three strains of *Trichomonas* (*T. hominis*, *Trichomonas* sp. from *Macacus rhesus* and *Trichomonas* sp. from *Macacus nemestrinus*), which had been under cultivation and observation for long periods without any cysts being found, in order to discover whether the free flagellate could withstand the concentration of hydrochloric acid ($N/20$) present in the normal human gastric juice. From the experimental evidence obtained it was concluded that, if infection is brought about in man or macaque by swallowing the free flagellate, passage through the stomach of a

[1] A study begun during my tenure of a Beit Memorial Fellowship and completed since I became a Yarrow Fellow.

physiologically normal individual must be rapid and infection due to the survival of a few trichomonads, or possibly of only one. Dobell (1931) has since infected a human being with a culture of *Trichomonas* from a macaque.

The following experiments were made in order to discover whether the free flagellate of *Trichomonas batrachorum* can pass through the stomach of an amphibian and set up infection in the gut, and also, as no distinguishing morphological difference has been found (Bishop, 1931), whether *Trichomonas batrachorum* in *Rana temporaria* and *Bufo vulgaris* is one species.

II. THE EFFECT OF RAIN WATER UPON CULTURES OF *TRICHOMONAS BATRACHORUM*

Trichomonas batrachorum is the protozoan parasite found most frequently in *Rana temporaria* and *Bufo vulgaris*. It often occurs in large numbers, and even in cases where it is so scarce that it is not seen on direct examination of the faeces its presence can almost always be demonstrated in cultures made from the apparently negative material. As no authentic cyst is known the free flagellate is presumably the source of infection. If this is so it must be very resistant to conditions outside the body of the host and be able to live for some time in water, as under natural conditions tadpoles must become infected by swallowing the flagellate in water contaminated by faeces. Ninety per cent. of the tadpoles which I have examined in the last three years have been infected with *Trichomonas batrachorum*.

Observations have been made upon the effect of water upon *Trichomonas* from man. Kofoid and Swezy (1923) found sterilised tap water, rain water, and creek water less favourable for the survival of *Pentatrichomonas ardin delteili* than the undiluted stool, and that the flagellates disappeared from such dilutions in three days. Hegner (1928), experimenting with dilutions of faeces containing *Trichomonas hominis* in tap and distilled water, found that the diluent had an adverse effect upon viability, and he attributed this chiefly to changes in the osmotic pressure of the medium. Kuczynski (1914) states that he saw trichomonads in pools in which were droppings from cows and sheep. Cleveland (1928) described a *Trichomonas* (*Tritrichomonas fecalis*) from man which grew at 18–23° C. in faeces diluted with tap water, and in hay infusion.

The following experiments were made in order to study the effect of rain water upon *Trichomonas batrachorum* in culture. The strain of *Trichomonas* used (*strain* T.I.) was isolated from a toad on 9. iv. 31 and cultivated in test-tubes on a medium consisting of an inspissated horse-serum slope covered with inactivated horse-serum diluted 1: 10 in 0·5 NaCl solution, to which a little solid sterile rice starch was added before inoculation. Repeated examinations were made of the subcultures at all ages but no cysts were ever found.

Approximately 1 c.c. of a subculture of *strain* T.I. was dispersed in a Petri dish containing sterile rain water to a depth of 0·5 cm. Within forty-eight hours only dead trichomonads were found, and all cultures inoculated from this dish

on or after this time were negative for *Trichomonas*. This experiment was repeated six times with different subcultures of *strain* T.I. and the trichomonads were, in every case, in a dying condition after twenty-four hours and quite dead after forty-eight hours. Six experiments were then made in which approximately 1 c.c. of culture of *Trichomonas batrachorum* (*strain* T.I.) was put into a test-tube containing a column 9 cm. in height of sterile rain water. The tubes were examined at first every second day but later at weekly intervals.

Table I. *Length of survival of* Trichomonas batrachorum (strain *T.I.*) *in test-tubes containing columns of sterile rain water* 9 *cm. in height.*

No. of subculture	Date of dilution	Date of death
T. 7	15. v. 31	16. x. 31
T. 10	28. v. 31	27. x. 31
T. 17	13. vii. 31	22. xii. 31
T. 19	13. vii. 31	20. vii. 31
T. 18	20. vii. 31	22. vii. 31
T. 21	8. viii. 31	27. viii. 31

The trichomonads were normal in structure but became much smaller than in culture. Although at the beginning they were well mixed in the liquid they soon sank to the bottom of the tubes and remained there. Four experiments similar to those in Table I were made and the tubes examined in 14 days. As they were positive for trichomonads they were tested with methylene blue which was found to be completely reduced for 3 cm. from the bottom of the tubes. Absence of oxygen was evidently one of the chief factors in the greater length of time of survival of *T. batrachorum* in the deep tubes compared with those in the Petri dishes. From Table I, however, it will be seen that though the flagellates were treated similarly in each experiment in the deep tubes the length of survival ranged from two days to five months. Dilution with rain water, though sterile, probably alters the balance of bacteria present, and the rapid growth of unfavourable bacteria would annihilate the trichomonads.

Escomel (1913) states that he found *Trichomonas intestinalis* in drinking water. Later (1925) he says that he found *T. batrachorum* in water in which *Telmatobius gebski* and *Bufo spinulosus* lived. He claims to have sterilised *Telmatobius* by starvation for eight days followed by a diet of bread crumbs, and to have infected them with *Trichomonas hominis*.

I have repeatedly tried to grow *T. batrachorum* at 37° C. but have never succeeded. The trichomonads invariably died in a few hours. All individuals in very rich cultures were dead after incubation at 37° C. for twelve hours. My attempts to grow a strain at 30° C. were also unsuccessful though the flagellates remained alive for a few days. Cleveland (1928) found that *Tritrichomonas* from *Rana pipiens* would not grow in culture at human body temperature. Whereas, in my experience, *Trichomonas hominis* will live for days at room temperature (15–20° C.) it will not multiply. There seems to be considerable difficulty, therefore, in regarding Amphibia as a possible host and reservoir of infection for *T. hominis*.

III. INFECTION EXPERIMENTS WITH FROG AND TOAD TADPOLES

In order to infect animals experimentally with intestinal Protozoa it is necessary that the animals used should be free from such Protozoa. Dobell (1918) reared tadpoles free from Protozoa, and his technique has been adopted here. On 8. iii. 33 a pair of frogs, clasped ready for spawning, were pithed, the skin of each individual washed with 70 per cent. alcohol, and the body cavity opened. The eggs were removed, with precautions against contamination from the skin and gut, and spread out in sterile crystallising dishes. They were fertilised with sperms taken, with similar precautions against contamination, from the vesiculae seminales and teased up in 0·5 NaCl solution. The dishes were partly filled with tap water sterilised by a Seitz filter. The remains of the jelly was removed when the tadpoles had hatched, as its decay leads to their death. Dobell fed the tadpoles upon human faeces and found that they flourished upon the diet. The present tadpoles were fed upon boiled dog biscuit and human faeces. The faeces were from a person who, during the last five and a half years, had been examined frequently for intestinal Protozoa, both by direct microscopic examination of faeces and of cultures made from it, with invariably negative results. For the fortnight preceding the first feeding of the tadpoles daily examinations of faeces and cultures inoculated from it were made and were negative, and similar examinations of samples used for feeding during the course of the experiments were also negative.

Three strains of *Trichomonas* were used and each strain contained *Trichomonas* alone, together with a mixed growth of bacteria; and although examined at all stages of the subcultures cysts had never been found. *Strain* T.II. was isolated from a toad three months prior to the beginning of the experiments, and grown, at room temperature (16–20° C.), on the medium described above (p. 27) for *strain* T.I. *Strain* F. was isolated from a frog ten weeks before the beginning of the experiments, and grown under the same conditions as *strain* T.II. *Strain* L. was isolated, ten weeks before use, from a lizard (*Lacerta viridis*), and grown in the liquid medium alone but with a little of the solid sterile rice starch. This *Trichomonas* had three flagella and agreed in structure with *T. lacertae* Prowazek as illustrated by Wenyon (1920) for specimens from *Lacerta agilis* and *Agama stellio*.

Before feeding tadpoles with such cultures of *Trichomonas* it was necessary to remove as much serum as possible from the flagellates by sedimenting them in 0·5 NaCl solution, or in sterile tap water, as the serum proved toxic to the tadpoles. The trichomonads sink to the bottom of the tube and the supernatant fluid can then be removed.

Exp. I. Tadpoles (*Rana temporaria*) fed with *Trichomonas batrachorum* from *Bufo vulgaris* (*strain* T.II.).

On 17. iv. 33 six tadpoles were killed and the entire gut of each teased up and examined separately. All were negative for Protozoa. Six more examined on 21. iv. 33 were also negative. The tadpoles to be fed were put into a flask

containing sterile tap water and an equal number were put into a similar flask as controls, both sets being taken from the same dish as the ones which had been examined. The controls were fed on the ordinary diet but to the others was given a culture, washed by sedimentation, of *Trichomonas* (*strain* T.II.). The tadpoles immediately began to eat the culture sediment. To prevent confusion in examination with trichomonads not ingested and free in the water the tadpoles were removed after an hour and washed well in sterile water and put into a clean flask. The tadpoles were killed and examined at intervals and the whole gut teased up and examined. Twenty-four hours after feeding one tadpole was found negative. Three days after feeding two tadpoles were examined and were positive. The remaining three were examined five days after feeding and all were positive. The controls were all negative. On 3. v. 33 this experiment was repeated with five tadpoles, and the same number as controls, using the same strain of *Trichomonas* but a later subculture. One tadpole was examined a few hours after feeding and was well infected. The remaining four were examined, one two days after feeding, one five days after feeding and the remaining two seven days after feeding. All were well infected, and the controls were negative.

Exp. II. Tadpoles (*Rana temporaria*) fed with *Trichomonas* sp. from lizard (*strain* L.).

On 28. iv. 33 a similar experiment was performed using the *strain* L. of *Trichomonas*. Six tadpoles were fed, two were examined four days after feeding and the remaining four six days after feeding. Both they and the controls were negative. Many dead trichomonads were found in the gut of the tadpoles which had been fed with the culture.

Exp. III. Tadpoles (*Bufo vulgaris*) fed with *Trichomonas batrachorum* from *Bufo vulgaris* (*strain* T.II.).

Toad tadpoles were obtained, by the method described above for frogs, from a pair of toads killed and dissected on 20. iii. 33. As a vesicula seminalis is absent in *Bufo vulgaris* it is necessary to tease up the testes to obtain spermatozoa. On 19. iv. 33 six tadpoles, from one dish, were killed and examined and found negative for Protozoa. On 24. iv. 33 six tadpoles from the same source were fed with a culture of *Trichomonas* (*strain* T.II.) and six used as a control. Two days later they were killed and examined. Of the six fed with *Trichomonas* five were infected, and all the controls were negative. Later (18. v. 33), fourteen toad tadpoles were fed with a later generation of *strain* T.II. and on examination six days later all were infected, some heavily, but all the controls were negative.

Exp. IV. Tadpoles (*Bufo vulgaris*) fed with *Trichomonas* from the frog (*Rana temporaria*) (*strain* F.).

Six tadpoles were fed (24. iv. 33) with *Trichomonas* from the frog (*strain* F.). On examination two days later three were infected. One tadpole died. The remaining two were examined five days later and were negative. The controls were all negative.

This experiment was repeated (28. iv. 33) and the tadpoles examined two and five days later. All were infected and the controls negative.

Exp. V. Tadpoles (*Bufo vulgaris*) fed with *Trichomonas* sp. from the lizard (*strain* L.).

Six tadpoles were fed with a culture of *Trichomonas* from the lizard (*strain* L.). On examination six days later both they and the controls were negative.

IV. Infection experiments with *Salamandra maculosa*

In February 1932 three pregnant salamanders (*Salamandra maculosa*) were killed and the larvae removed from the oviducts with every precaution against contamination. The vitelline membrane, in which every larva is individually enclosed, was dissected away, and the liberated larvae transferred to sterile rain water. The larvae were fed with shreds of meat cut from the inside of a fresh piece of steak. They grew well. After three weeks twelve were killed and examined for Protozoa and were found negative. Cultures were made, using the part-liquid part-solid medium described above, from the intestinal contents of six others and these also were negative. Eight larvae were fed (24. iii. 32) with shreds of meat dipped in a rich culture of *Trichomonas batrachorum* (*strain* T.I. 37th subculture) (p. 27) from a toad. Twelve days after feeding three of the larvae were killed and examined. Two were negative for Protozoa and the third was heavily infected with *Trichomonas*. The following day the remaining five young salamanders were killed and examined. Three were negative but two were well infected with *Trichomonas*. Dividing trichomonads were found in the gut of one. The control larvae were negative. Later (12. iv. 32) eight more salamander larvae were fed with meat dipped in the same strain of *T. batrachorum* but in the 40th subculture. These larvae were killed and examined at intervals during the following twenty-eight days and all were heavily infected with a *Trichomonas* indistinguishable morphologically from the *T. batrachorum* in culture. The trichomonads were found in the rectum only. Controls, killed and examined at the same time, were negative.

V. Discussion

The results of the above experiments upon tadpoles of *Rana temporaria* and *Bufo vulgaris* indicate that it is possible to infect tadpoles of these species reared free from Protozoa with cultures of the free flagellate of *Trichomonas batrachorum*. Experiments upon the effect of rain water on cultures of *T. batrachorum* had shown that, whereas, in shallow water they die in forty-eight hours, in deeper water they can live for long periods. It has therefore been quite easy to feed tadpoles upon cultures of *T. batrachorum* added to the water. Tadpoles eat decaying organic matter and it is to be presumed that, in nature, they become infected with *T. batrachorum* by eating faeces deposited by adults or by previously infected tadpoles.

Experimentally frog tadpoles can be infected with cultures of *T. batrachorum* from toads, and conversely toad tadpoles with cultures of *T. batra-*

chorum from frogs. As there is no morphological difference between the trichomonads of frogs and toads to regard them as the same species is justified. *Trichomonas* from *Lacerta viridis* will not infect tadpoles of *Bufo vulgaris* or *Rana temporaria*. That they are not merely passed out of the host and unable to establish themselves mechanically, but die in the gut of the tadpole, was shown by finding many disintegrating trichomonads in the rectum of the tadpoles.

Salamandra maculosa can be infected experimentally with the free flagellate of *Trichomonas batrachorum* in culture. It was not possible to keep larvae alive until they became adult and so to ascertain if at that time they could also be infected with this flagellate. Accounts of the Protozoa in the gut of *Salamandra maculosa* give no description of a trichomonad comparable with *Trichomonas batrachorum*. *T. prowazeki*, described by Alexeieff (1909; 1910) from *Salamandra maculosa*, *Triton cristatus*, *Alytes obstetricans* and *Box salpa*, differs from *Trichomonas batrachorum* in having four flagella of unequal length. I examined eight mature *Salamandra maculosa*; they were slightly infected with *Trichomonas prowazeki* but *T. batrachorum* was not present. The rectum of each specimen was heavily infected with *Proteromonas longifilia*.

VI. Summary

1. Cysts of *Trichomonas batrachorum* have never been found in repeated examinations of faeces nor of cultures made from them from *Bufo vulgaris* and *Rana temporaria*.

2. In dilutions of cultures in rain water with large surfaces exposed to the air *Trichomonas batrachorum* dies in forty-eight hours; but in long columns of water and absence of oxygen they live, in some cases, for months.

3. *T. batrachorum* will not live at 37° C., and *T. hominis*, though it lives at room temperature, will not multiply.

4. Tadpoles of *Rana temporaria* and *Bufo vulgaris* and larvae of *Salamandra maculosa* have been reared free from Protozoa.

5. Tadpoles of all three species of Amphibia have been infected with *Trichomonas* by feeding them on cultures free from cysts.

6. Tadpoles of *Rana temporaria* can be infected with *Trichomonas batrachorum* isolated from *Bufo vulgaris*, and tadpoles of *B. vulgaris* with *Trichomonas* from *Rana temporaria*. It is concluded therefore that *Trichomonas batrachorum* in frogs and toads is one true species.

7. *Trichomonas* sp. isolated from *Lacerta viridis* will not infect tadpoles of *Bufo vulgaris* and *Rana temporaria*.

8. Cultures of *Trichomonas batrachorum* from *Bufo vulgaris* will infect larvae of *Salamandra maculosa* though this species of *Trichomonas* has not been described from this amphibian.

I am deeply indebted to Mr Clifford Dobell, F.R.S., for his helpful criticism of this paper.

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ON SOME GREGARINES FROM THE PRAWN, *PARAPENEOPSIS SCULPTILIS* (HELLER)

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(With 23 Figures in the Text)

Many species of cephaline Gregarines have been previously described from hosts belonging to widely different orders of Crustacea. These parasites are referred to the families Cephaloidophoridae Kamm, Gregarinidae Labbé, Porosporidae Léger, and Didymophyidae Léger. Kamm (1922) gave a list of these parasites, instituted the new family Cephaloidophoridae, and gave a description of all the species, except those referrable to Porosporidae, as till that time it was generally believed that Porosporidae were Schizogregarines. She fully discussed the grounds for placing the genus Cephaloidophora Mawrodiadi in a family by itself, and transferred to this genus several species previously described under *Gregarina* or *Frenzelina*.

Porosporidae are Gregarines which show an alternation of hosts between a decapod Crustacean and a Lamellibranch or a gasteropod Mollusc. In the decapod they grow as intestinal parasites to comparatively large size, and encyst either singly or as two or three individuals, and in these cysts are formed the so-called "gymnospores" with many buds. As the buds in the cyst do not copulate with one another, the phenomenon was regarded as schizogony and the family placed among the Schizogregarinidae. Recent researches of Léger and Duboscq (1925) and Hatt (1927, 1931) have shown, however, that the buds develop into gametes in the body of the second host. Reichenow (1929) accordingly placed the family among Cephalina.

The decapod Crustacea so far examined for their gregarine parasites have been lobsters, crabs or hermit-crabs. No prawns have so far been examined. We have studied the parasites from a prawn from Bombay, which was kindly identified by Dr B. N. Chopra of the Zoological Survey of India as *Parapeneopsis sculptilis* (Heller). This is known to occur fairly commonly all along the Indian coasts. Sporonts belonging to two distinct types have been met with, which can be referred to the family Gregarinidae. Both show bending and gliding movements and form interesting associations in simple or bifurcated or more complex chains, but cysts of only one kind have been found. These belong to the first type, which is provisionally referred to the genus *Hirmocystis*. The

second type is provisionally placed in the genus *Protomagalhaensia*. One or the other species is more numerous in different individual hosts, though in some both are equally represented. We are aware of the fact that unless the complete life history of a crustacean parasite is known, it cannot be assigned to a particular genus with a reasonable assurance of permanence, but we think it desirable to publish such observations as we have made, leaving the correct position of the species to be determined in the light of fuller knowledge later on.

In addition to the two gregarine species, cysts of a species of *Aggregata* have also been found, and are described in the sequel.

Genus **Hirmocystis** Léger 1892

Sporonts in associations of two to twelve (or more), in linear series, sometimes bifurcated or trifurcated. Epimerite a small conical or cylindrical knob,

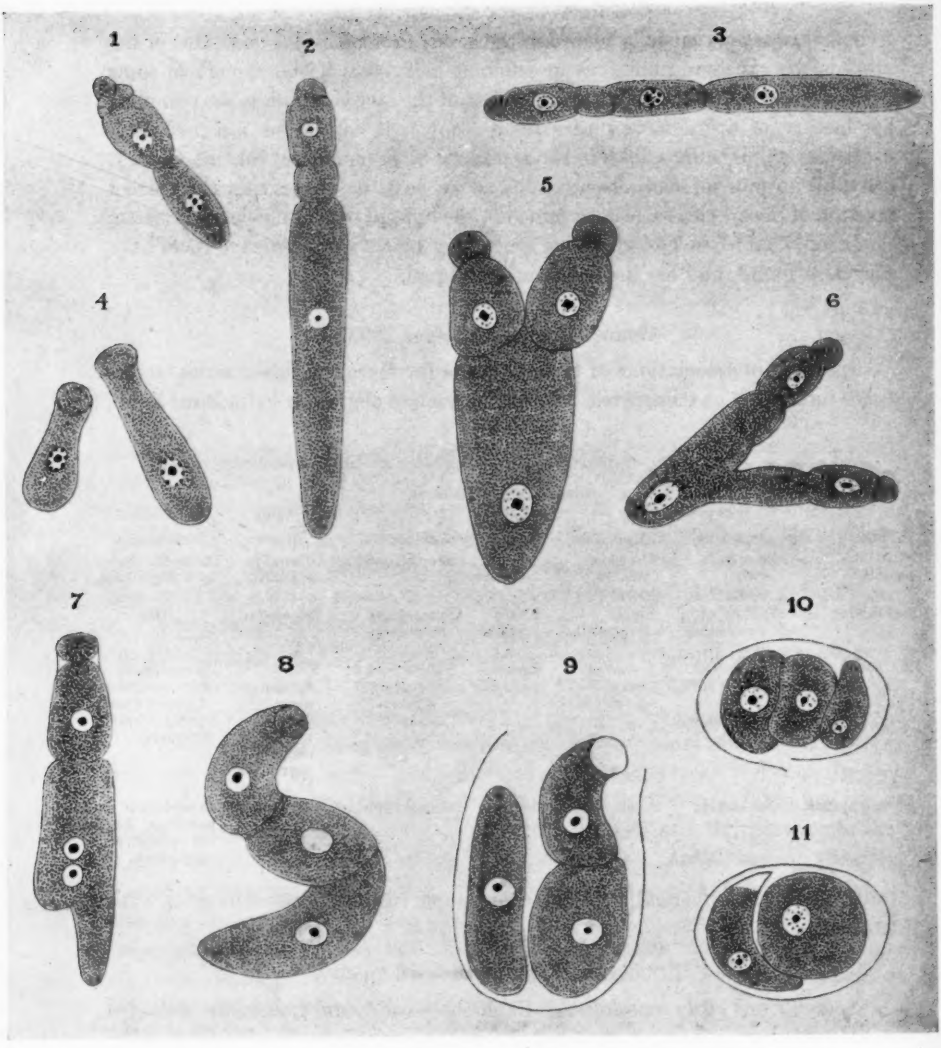
Table I. *Giving the characters of the families of cephaline Gregarines found in Crustacea.*

Family	Sporonts	Epimerite	Development	Cyst dehiscence	Spores	Remarks
Cephaloidophoridae	Two, from early syzygies	Absent or weakly developed	Intracellular	Simple rupture	Ovoidal or spherical	Entire life cycle in a single host.
Gregarinidae	Solitary or in associations up to 12	Simple, symmetrical	Extracellular	Spore-ducts or simple rupture	Ellipsoidal	Do
Porosporidae	Association of two or three individuals	Absent or weakly developed	Extracellular	Simple rupture setting free "gymnospores"	Naked sporozoite directly from a zygote or single sporozoite in a spore	Alternation of hosts. Copulation of gametes and sporogony in molluscs.
Didymophyidae	Association of two or three individuals	Small, simple papilla	Extracellular	Simple rupture	Ellipsoidal	No septum in satellites. Entire life cycle in a single host.

caducous. Cysts spherical, dehiscence by simple rupture. Spores ovoidal, with two coats.

Hirmocystis (?) **parapeneopsisi** sp.nov.

Sporonts and early associations. Cephalonts are found free in the gut. No individuals have been observed attached to the epithelial cells of the gut of the host. The sporonts form early associations, even when the primite is still retaining its epimerite. In one such association (Fig. 1) the epimerite is a small rounded knob, the primite and satellite are equal in length and measure 44·4μ each. The protomerite of the primite is hemispherical, flattened against the deutomerite; the deutomerite is bulging much more, its maximum width being 18·5μ. The length of the protomerite : total length of the primite :: 2 : 11; the width of the protomerite : width of the deutomerite :: 3 : 5. The protomerite of the satellite is more or less quadrilateral, with parallel edges, and distinctly marked off by a septum. The maximum width of the satellite is



Figs. 1–11. *Hirmocystis* (?) *parapeneopsisi*.

Fig. 1. Early association, primite still retaining its epimerite.
Fig. 2. Chain of two individuals.
Fig. 3. Chain of three individuals.
Fig. 4. Sporonts.
Fig. 5. A new type of association, showing two primites followed by a single satellite.
Fig. 6. The single satellite has been torn asunder owing to the two primites pulling in contrary directions.
Fig. 7. Two satellites running into one another and showing complete fusion.
Fig. 8. Association of three individuals, the chain being curved up.
Figs. 9, 10. Oval cysts containing three individuals.
Fig. 11. Cyst containing two individuals.

12·2μ. While in these early associations the primite usually measures about 40μ, the length of the satellite is usually much more, in one case as much as 166·5μ (Fig. 2). Sometimes chains of three individuals are also met with (Fig. 3). In such cases the first satellite is longer than the primite, and the second satellite longer than the first. The septum is usually present in the first satellite, though sometimes it may be wanting; it is always absent in the second satellite. The terminal satellite is rounded at its end. The nucleus is spherical and 7–9μ in diameter. The nuclear membrane is thin and there is a single karyosome. In the younger sporonts, there are a number of prominent chromatin particles adhering just within the nuclear membrane.

In some cases individual sporonts are met with, which have lost their epimerites, and are not forming syzygies (Fig. 4). These measure 40–80μ in length and 14–18μ in their maximum width. The protomerite is more or less spherical and relatively large. The deutomerite is somewhat constricted in its anterior part, and widest behind. The length of the protomerite : total length of the primite :: 1 : 5·5 or 7; the width of the protomerite : width of the deutomerite :: 3 or 4 : 5.

Unique associations. A few cases of a unique type of association (Fig. 5) have also been met with, in which two primites are followed by a single satellite. So far as we know, such an association has never been described or figured before for any Gregarine. The primites are posteriorly rounded off and fit into curved depressions along the anterior margin of the satellite. The satellite is about twice as long as each primite, and bluntly tapers posteriorly. The nucleus of the satellite is larger and irregularly rounded. On other occasions the primites are larger than the satellite. Sometimes one comes across specimens in which two satellites run into one another, and show partial or complete fusion (Figs. 6 and 7), and the deutomerite may contain one or two nuclei.

Associations of larger individuals. Linear syzygies of two or three very much larger individuals are more frequently met with. In these the primite may measure anything from 74 to 203μ in length, and the satellite from 92·5μ to 222μ. In the primite, the length of the protomerite : total length of the primite :: 1 : 7 to 11; the width of the protomerite : width of the deutomerite :: 1 : 1·8 to 2. The diameter of the nucleus varies from 11 to 16·6μ. The greatest width of the primite is at about one-third of its length from the posterior end, and of the satellite in its anterior third. The septum of the first satellite also is generally absent in these syzygies of larger individuals. If a second satellite is present, its septum is always wanting. The posterior end of the terminal satellite is narrow and rounded. Many of these large sporonts clearly show longitudinal epicytal striations. On some individuals the myonemes may be seen as large deeply stained dots situated along the edge of the endoplasm, and the network of fibrils can also be made out. The endoplasm is granular.

Cysts. Besides the numerous straight chains of sporonts, associations are met with (Fig. 8) in which the chain is curved up. This condition is followed by encystment. Oval cysts are seen (Figs. 9 and 10) containing usually three

individuals, the protomerite of the primite being still recognisable, as it is more transparent. Sometimes (Fig. 11) the cyst contains only two individuals lying side by side. Inside the cyst, the sporonts give rise to gametes. The gametes are small, oval bodies, which are similar in size and form. We have entirely missed the cysts containing the spores, and the free spores, and so nothing is known about the dehiscence of the cysts, and the form of the spores.

Diagnosis. Sporonts stoutly built, forming early syzygies. Associations of two or three individuals in linear chains, or unique associations of two primites with a single satellite. Maximum length of association 425μ, maximum width 44μ. Free sporonts 40 to 80μ. Ratio, length of protomerite : total length :: 1 : 5 to 7; width of protomerite : width of deutomerite :: 1 : 1·4. Epimerite small rounded knob. Protomerite hemispherical or flattened, constricted at the septum, deutomerite cylindrical or barrel-shaped, broadly rounded or flattened behind. Satellite longer than the primite, second satellite longer than the first. Nucleus spherical, with a single karyosome. Cysts spherical, or slightly elliptical. Dehiscence of cysts and spores not observed.

Habitat. Intestine of *Parapeneopsis sculptilis* (Heller): taken at Bombay.

Systematic position. All the Gregarines previously known from decapod Crustacea belong with one single exception to Cephaloidophora or *Porospora*. The Gregarine now under consideration does not belong to either of these genera. It does not belong to the first as the trophozoite is not intracellular, and the associations are not always of two sporonts only. It does not belong to *Porospora*, as "gymnospores" bearing radially arranged buds are not developed in the cyst. Its position is clearly among the Gregarinidae. Of the many genera belonging to that family, only two, viz. *Uradiophora* and *Pyxinoides*, are previously known from crustacean hosts. In both these, associations are of two sporonts, and the epimerites are of special types. The form resembles *Hirmocystis* inasmuch as sporonts form associations of two or more, and the epimerite is small, knob-like and caducous. But the species cannot be definitely placed in that genus until the dehiscence of the cyst and the form of the spores has been observed.

Genus **Protomagalhaensia** Pinto 1918

Sporonts in associations of several individuals, often attached laterally. Sporonts attenuate. Myonemes prominent. Epimerite and cysts unknown. Spores barrel-shaped, with a spine at each corner.

In addition to the above diagnosis as quoted from Kamm (1922), Pinto (1923) mentions in the diagnosis of the genus that the development is always intracellular, and that in the syzygies the protomerite of the satellite embraces

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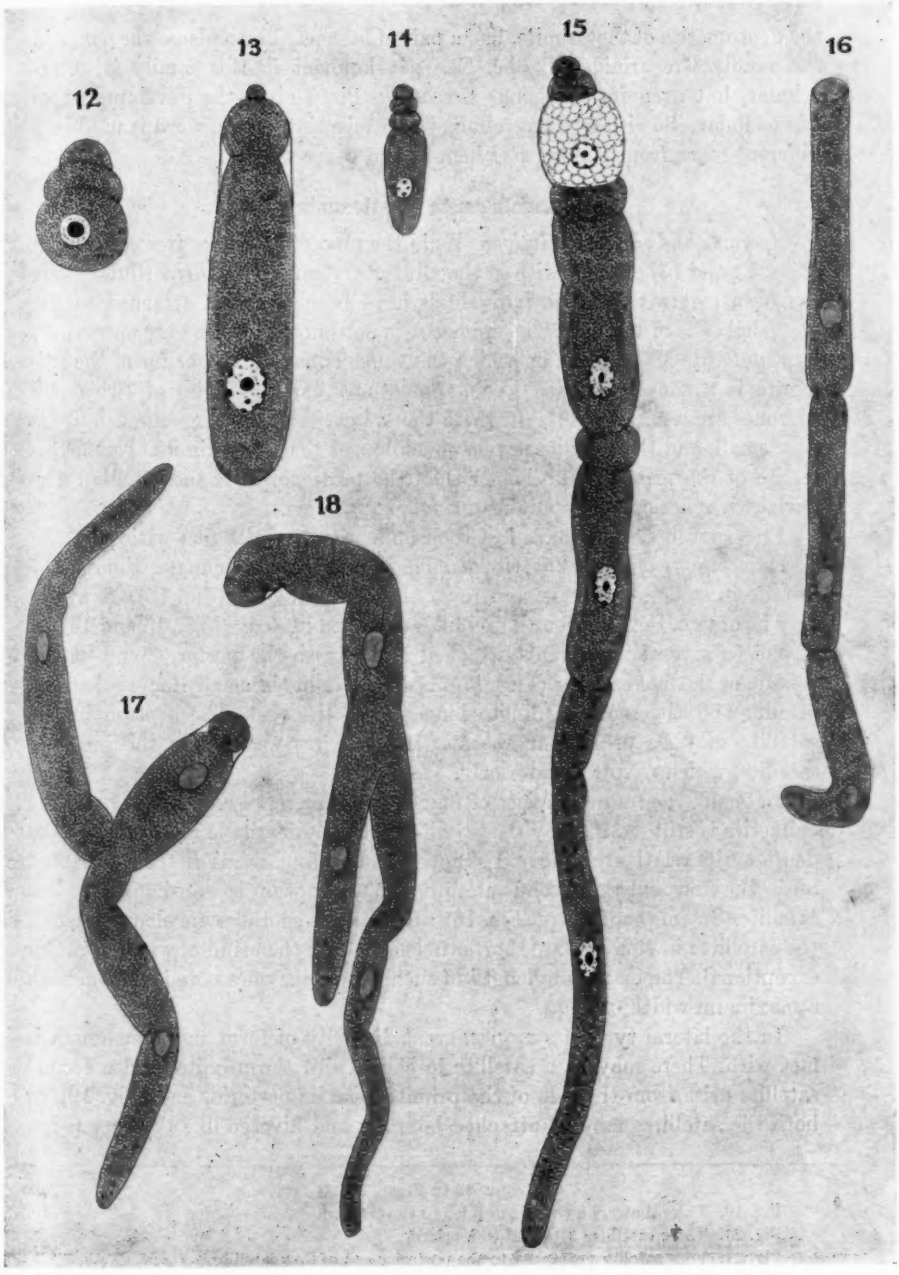
Legends to Figs. 12–18

Figs. 12, 13. Free cephalonts.
Fig. 14. Early syzygy.
Figs. 15, 16. Linear syzygies.
Figs. 17, 18. Lateral attachment of satellites to the primite.



Figs. 12–18. *Protomagalhaensia* (?) *attenuata*.

the deutomerite of the primite, like a pair of forceps. Pinto places the genus in the family Gregarinidae Labbé. The development in this family is extracellular, but even for the genus *Gregarina*, Pinto gives the development as intracellular. So either "intracellular" is a misprint, or the word is used in a different sense from Kamm, Reichenow, and other writers.

Protomagalhaensia (?) attenuata sp.nov.

Sporonts and early associations. As in the preceding species free cephalonts (Figs. 12 and 13) are met with in the alimentary canal. No intracellular stage has been met with and no individuals have been observed attached to the epithelial cells of the host. The epimerite is not known for the only species, *P. serpentula* (de Magalhaes), on which the genus is based. In our form, the epimerite is a simple rounded knob. Sometimes early syzygies of two small sporonts are seen (Fig. 14). In these the epimerite is wanting, the primite is very small, and the satellite is very much longer than the primite. The deutomerite of the primite is no longer than the protomerite of the satellite. The nucleus is spherical and contains a single karyosome.

Free sporonts which have lost their epimerites are also met with.

Associations of larger individuals. The sporonts are attenuate, though the primite in some syzygies is seen to be short and bulging (Fig. 15). Syzygies may be of two, three, or four individuals arranged linearly (Figs. 15 and 16), or of one to several individuals attached laterally to the posterior end of the primite or the first satellite (Figs. 17–21). The satellite is usually longer than the primite and the second satellite longer than the first. The septum in the satellite may be present or absent. It is always wanting in the terminal satellite, which narrows posteriorly.

In one of the linear syzygies of four individuals represented in Fig. 15, the epimerite is still retained by the primite. The protomerite is rounded, and the deutomerite relatively wide and gibbous. The protomerite is distinctly marked off in the first and the second satellite, but the septum is wanting in the last satellite. In another syzygy (Fig. 16), all the three sporonts are elongated, and the satellites in this case are shorter in length than the primite, which is rather exceptional. The maximum length of such simple syzygies noted is 473μ, with a maximum width of 24μ.

In the lateral type of syzygies, great diversity of form and attachment is met with. There may be a satellite in a line with the primite, and a second satellite arise from the side of the primite near its posterior end (Fig. 19), or both the satellites may be attached laterally and diverge like two legs (Figs.

Legends to Figs. 19–23

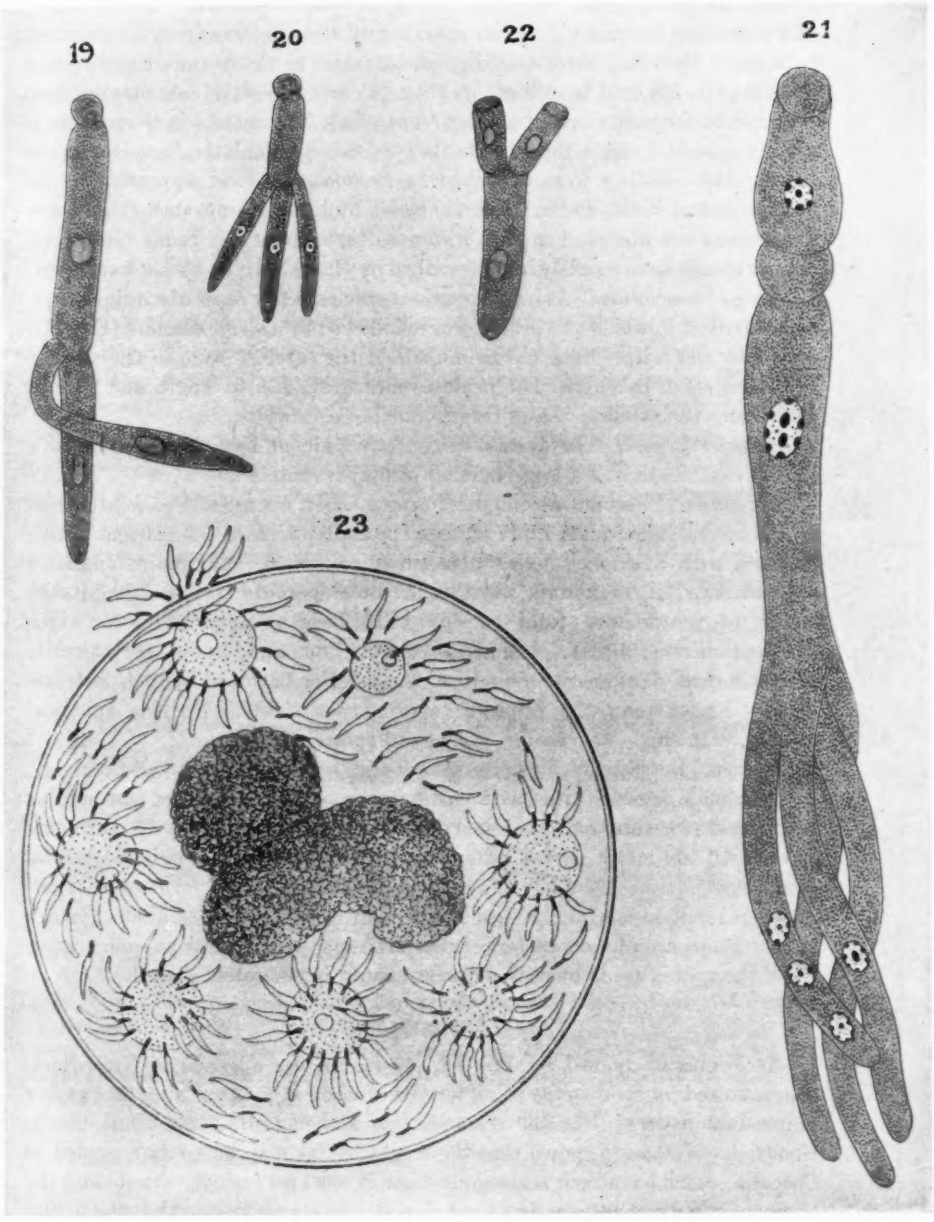
Fig. 19. Lateral attachment of satellites to the primite.

Fig. 20. Three satellites attached to a primite.

Fig. 21. Four satellites attached to the posterior end of first satellite.

Fig. 22. A new type of association showing two primites followed by a single satellite.

Fig. 23. *Aggregata* sp. Cyst with merozoites.



Figs. 19–22. *Protomagalhaensia* (?) *attenuata*.
Fig. 23. *Aggregata* sp.

17 and 18). The satellites may be equal or unequal in size and variously bent, and there may be no dividing line marking off the satellites from the primite. Sometimes there are three satellites all attached to the posterior end of the primite (Fig. 20), and in still others (Fig. 21) several individuals may be seen attached to the posterior end of the first satellite. The nucleus in the primite is usually spherical, and in the satellite always oval. Similar lateral associations of two to five satellites were described in *Protomagalhaensia serpentula* by de Magalhaes and Pinto, and in *Gregarina rigida* by Ellis. Kamm states that these phenomena are observed in rare instances throughout the genus *Gregarina*. Similar chains have recently been described by Hatt (1931) in *Nematopsis legeri*.

Unique associations. As in the previous species, a few cases of a unique type of association, in which two primites were followed by a single satellite (Fig. 22), were also met with. Both the primites and the satellite were of the slender type, and small in length. The primites measuring 22μ in length and $7{\cdot}4\mu$ in width, and the satellite 37μ in length and $11{\cdot}1\mu$ in width.

Cysts and spores. The cysts and spores could not be found in spite of a careful examination of a large number of preparations.

Diagnosis. Sporonts attenuate, forming early syzygies. Associations of two to several individuals, often attached laterally. Unique associations of two primites with a single satellite sometimes met with. Maximum length of association 473μ, maximum width 22μ. Free sporonts 20μ to 70μ. Ratio, length of protomerite : total length :: 1 : 13; width of protomerite : width of deutomerite :: 1 : 1·1. Epimerite simple rounded knob. Protomerite hemispherical, deutomerite cylindrical, rounded or flattened behind. Satellite usually longer than the primite, tapering posteriorly. Nucleus spherical or oval, with single karyosome. Cysts and spores not known.

Habitat. Intestine of *Parapeneopsis sculptilis* (Heller): taken at Bombay.

Systematic position. As the development is extra-cellular and associations are formed of several individuals, the form belongs to the family Gregarinidae Labbé. Of the many genera belonging to that family, the species resembles *Protomagalhaensia*, inasmuch as the sporonts are attenuate, form associations of several individuals, and lateral attachment of sporonts is common. Spores in that genus are of a characteristic barrel-shape, with a spine at each corner. Until the spores are known, the species cannot be definitely placed.

Aggregata sp.

As originally defined by Frenzel, *Aggregata* was a genus of Gregarines, characterised by sporozoites being formed directly in the cyst round a number of residual masses. But the researches of Dobell (1914, 1925) and Pixell-Goodrich (1914) have shown that the Aggregatidae may be safely regarded as Coccidia, which have their schizogony stage in one host (usually a crab) and the sporogony in another (usually a Cephalopod). It is now known that the bodies produced in the cyst are merozoites and not sporozoites as was formerly supposed.

Along with the two species of Gregarines described above, a large number of cysts (Fig. 23) were found throughout the intestine of the prawn. These cysts are visible to the naked eye as minute specks. Cysts are spherical or slightly oval and measure 193–246μ in diameter. The length and breadth of a number of cysts are noted below (in microns):

	Length	Breadth
(1)	193	193
(2)	193	184
(3)	211	184
(4)	211	176
(5)	228	158
(6)	246	158

Each cyst has six or seven pear-shaped apertures through which merozoites escape. The merozoites are set free and are also found in the alimentary canal of the prawn. The merozoites are very characteristic bodies (Fig. 23). Each is a curved spindle-shaped body, with a broad central portion and pointed ends. The nucleus consists of deeply staining granules and occupies one end of the merozoite. The merozoites measure 11 to 16μ in length. When free, they are very active bodies. They move with sudden jerky or springy movements, and the nuclear end is usually anterior in these movements. The cysts and the merozoites are very similar to those of *Aggregata eberthi* found by Léger and Duboscq in the intestine of the crab *Portunus*, except that in the latter case the pear-shaped openings are not present.

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QUINANIL AS A BACTERICIDAL AGENT IN THE ISOLATION OF AN INSECT FLAGELLATE

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Introduction

The cultivation of flagellate parasites from the alimentary tracts of Diptera is difficult owing to the general presence of bacteria which rapidly overgrow the culture. Amongst the first records of successful isolation of pure cultures of Protozoa is that of Novy, MacNeal and Torrey (1907), who succeeded in isolating pure cultures of flagellates of the Trypanosomidae from the gut of *Culex pipiens* by plating dilutions of the gut contents on to blood agar plates. Nöller (1917) isolated *Leptomonas fasciculata* by similar methods. Drbohlav (1926) obtained *Herpetomonas muscarum* from *Lucilia* by plating on glucose-blood agar media of varying hydrogen-ion concentrations, whilst Noguchi and Tilden (1926) isolated a number of flagellates from insects and plants by simple plating on blood agar plates. Relatively few of the many attempts made have been successful. The use of bactericidal substances in attempted isolations of Protozoa has been tried by D. A. Koch (1926) with *Entamoeba gingivalis* but without success. Weiss and Weiss (1926) tried to purify cultures of *Spirochaeta pallida* by destroying contaminants with suitable chemicals and a number of investigators have used acriflavine to keep down the bacterial population in cultures of various intestinal Amoebae, of *Trichomonas* and *Balantidium* (Dobell and Laidlaw, 1926; Bishop, 1931; Jameson, 1927). Luck and Sheets (1931) sterilised cysts of the free living ciliate *Euplotes* by chemical treatment but excystation did not readily proceed after such treatment. To the best of our belief the following is the first record of a successful isolation of a pure culture of a flagellate from the gut of an insect as a result of the treatment of the gut contents with a bactericidal agent.

Material and methods

The flagellate employed was obtained from the Anthomyid fly *Polietes lardaria* Fallen, which is abundant near a certain dairy farm in the Keighley district. Keilin and Tate (1930) quote Portchinsky's statement that the larvae of this fly live entirely in the excrement of cattle. The flies were taken from June to September in a sunny porch, on bushes, or sunning themselves on stones. About 300 flies were examined during the summers of 1931 and 1932 and in each season the rate of flagellate infection approximated to 90 per cent. The parasites correspond in morphology, size and location with those described

by Mackinnon (1910) in certain dung flies. She considered the infections to be those of *Herpetomonas muscarum* Leidy, a flagellate that has been recorded from many genera of Diptera (Wenyon, 1926). We should have referred it without hesitation to this species but that its cultural requirements do not agree with those of the flagellates of *Calliphora* in some tests that have been made by two of us (F. C. H. and G. N. B.). It is of course possible that there are biological races of this species.

The leptomonas form of the flagellate occurs in great numbers in the midgut of the fly within the peritrophic membrane as far forward as the proventriculus. The leishmania forms are found in the rectum and in that site also occur the intermediate phases which characterise the shortening process. If the proventriculus of the fly is pulled away from the midgut it brings with it a long stretch of the peritrophic membrane with its contained flagellates. The parasites obtained in this manner are more readily handled than if derived from the teased up gut.

In the earlier attempts at isolation the peritrophic membranes were immediately suspended in sterile citrate-saline and ground up in a quartz mortar. The suspension was centrifuged for 5 minutes and the supernatant fluid which contained but few flagellates was rejected. The deposit was resuspended in fresh citrate saline and this process of washing was repeated several times. During the summer of 1932 attempts were made to cultivate the organism from such washed preparations using the following methods: (1) by plating on N.N.N. media, the plates being kept in sterile moist chambers; (2) by the inoculation of large series of fluid media with loopfuls of progressive dilutions of the suspension of the flagellate; (3) by inoculating further series of media with suspensions which were left standing for 2, 4, 6 and 18 hours respectively, rewashed, resuspended in saline in increasing dilution and finally inoculated; (4) by the inoculation of suspensions prepared as in (2) and (3) on to N.N.N. plates. In no single case were flagellates ever seen on subculture. Suspensions were next exposed to buffer solutions at *p*H 5·0, 6·0, 7·0 and 8·0 respectively but again without success.

On the advice of Mr Ellingworth we decided to try the action of quinanil, a powerful bactericidal substance described by Armitage, Gordon, Cohen, Ellingworth and Dobson (1929), and which according to Browning, Cohen, Ellingworth and Gulbranson (1929) possessed but little trypanocidal activity. Centrifuged deposits from the peritrophic membranes were mixed with equal volumes of quinanil solutions (concentration 1/500, 1/5000, 1/50,000). The mixtures were allowed to stand at laboratory temperature for periods of 1, 2, 6 and 18 hours, and subcultures were made into tubes of a meat extract medium prepared according to the formula of Anderson, Happold, McLeod and Thomson (1931) which had proved particularly favourable to cultures of *Leptomonas ctenocephali*. From an inoculation of a suspension on 1/1000 quinanil solution which had been left standing for 18 hours a culture of the flagellate resulted. This was thought to be pure, but a latent contamination

appeared which rapidly overgrew the culture. At this point the supply of infected flies ceased for the year.

During the winter further studies by two of us (G. N. B. and F. C. H.) on the effect of various bactericidal substances upon the growth of various leptomonads confirmed us in our choice of quinanil as a bactericidal substance likely to be favourable to our purpose. Our technique was modified and the flagellate was isolated at the first attempt in the spring of 1933. The method adopted was followed on several occasions with the same flagellate and it has repeatedly yielded pure cultures.

Method recommended

The peritrophic membranes were ground up in saline and lightly centrifuged for a short time to settle the grosser débris. The supernatant suspension containing most of the flagellates was then centrifuged at high speed and the deposit resuspended in sterile saline and divided into three equal portions. To one was added an equal volume of sterile saline, to another an equal volume of 1/50,000 quinanil and to the third an equal volume of 1/5000 quinanil. All three preparations were well mixed and a loopful of each was inoculated after 1, 2, 3 and 4 hours or more into Locke serum agar[1] plus quinanil to a concentration of 1/50,000, and Locke serum agar plus quinanil to a concentration of 1/10,000. The following is a summary of results from a large number of experiments.

In one instance a flagellate developed from a saline suspension inoculated in 1/50,000 quinanil Locke serum agar, the saline suspension having stood for 4 hours prior to inoculation. The culture was contaminated and though it showed dividing forms on the second day none was visible on the seventh day. None of the suspensions exposed to 1/100,000 or 1/10,000 quinanil solutions for one hour prior to inoculation into the three types of media produced cultures, but with longer periods of preliminary exposure a high percentage of positive cultures developed, many of which were shown to be free from bacterial contamination. It is however advisable as a general procedure to reinoculate the cultures of the flagellate into fresh media containing the bactericidal substance as soon as definite growth is established in case the effect has been bacteriostatic rather than bactericidal. Latent contaminations are thus not developed and most of the subcultures, if not all, are free from bacteria. We have taken as a criterion of purity the maintenance of our cultures free from demonstrable bacterial growth for over twenty generations in bouillon, and the failure of contaminants to develop on "chocolate" agar under aerobic and anaerobic conditions. A typical protocol of the results of one experiment is given in Table I.

The direct plating of saline suspensions on Nöller's medium of various *p*H adjustment was once successful. The plates were made up at a range of

[1] Locke solution 1000 c.c., melted clear agar 70 c.c., adjusted to *p*H 7·4, tubed in 4·5 c.c., autoclaved, 0·5 c.c. ox or rabbit serum added to each tube.

*p*H 5·5, 6·0, 6·5, 7·0, 7·5, 8·0. After incubation for 4 days at room temperature plates *p*H 7·5 and 6·5 showed growth of flagellates. Subcultures were made and proved to be free from bacteria. These subcultures were subjected to the tests for purity carried out as described above and were found to be quite free from bacterial contamination. The element of chance accounts for this one success among so many failures to obtain pure cultures without the use of quinanil.

We observed with interest that when we attempted to purify contaminated plate cultures of the flagellate from suspensions exposed to quinanil, and subsequently inoculated into media containing quinanil, isolation was never achieved if the period of preliminary exposure to 1/100,000 quinanil was less

Table I. *A typical protocol of one series of inoculations.*

Treatment of suspension	Medium inoculated	Result + = a light infection + + = a heavy macroscopic infection		
0·1 c.c. flagellate suspension + 0·1 c.c. 1/50,000 quinanil	*a* = no quinanil *b* = 1/50,000 *c* = 1/10,000	2 days	7 days	Further subcultures
2 hours	*a*	·	·	·
	b	+ +	+	·
	c	+ +	+	·
3 hours	*a*	·	·	·
	b	+ + Contam.	+ +	Contam.
	c	+ +	+ +	+ +
4 hours	*a*	·	·	·
	b	+	+ +	+ +
	c	+	+ +	+ +
0·1 c.c. flagellate suspension + 0·1 c.c. 1/5000 quinanil				
2 hours	*a*	·	·	·
	b	+ +	·	·
	c	+ +	·	·
3 hours	*a*	·	·	·
	b	+ +	+ +	+ +
	c	+ +	+ +	+ +
4 hours	*a*	·	·	·
	b	+ +	+ +	+ +
	c	+ +	+ +	+ +

than 4 hours. On the other hand in isolations effected direct from the fly, previous exposure of the flagellate suspension to 1/100,000 quinanil for 2 hours only was satisfactory.

The method advocated was also successfully used in the isolation of a flagellate in a single attempt from a *Calliphora* which was caught in the laboratory. As mentioned above the cultural characteristics of the flagellate obtained were not identical with those of the flagellate from *Polietes lardaria*, nor with those of *Herpetomonas parva* and *H. media* which Noguchi and Tilden (1926) isolated from *Calliphora* spp. This important point is receiving further consideration, and we do not bind ourselves at present to the specific identity of the flagellate from *Polietes lardaria*.

SUMMARY

A flagellate from *Polietes lardaria*, closely allied to *Herpetomonas muscarum*, has been repeatedly isolated in bacteria-free culture by the use of quinanil after numerous attempts to isolate it by other methods with one exception had failed. The flagellates were exposed to 1/10,000 or 1/100,000 quinanil for 2–4 or more hours and cultivated in Locke serum agar, the medium for the first subculture containing quinanil in concentrations of 1/10,000 or 1/50,000. The proofs of the purity of the cultures were exhaustive.

We are greatly indebted to Dr St John-Brooks and the National Collection of Type Cultures for certain of the flagellates isolated by Noguchi and Tilden. One of us (F. C. H.) has also been in receipt of grants from the Medical Research Council, and he would like to take this opportunity of expressing his thanks to that body.

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HISTORICAL REVIEW AND RELATIONSHIP OF THE GENERA OF THE FAMILY IXODIDAE

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(With 3 Figures in the Text)

Nuttall and Warburton (1911, pp. 105–13) give a detailed account of the systematic survey of the family Ixodidae and recognise nine genera in the family. They consider *Aponomma* Neumann as a subgenus of *Amblyomma* Koch, but I consider it a valid genus.

Neumann (1913, pp. 147–9) established *Pterygodes* as a subgenus of *Rhipicephalus* Koch. Schulze (1919, pp. 191, 192) established *Cosmiomma* and *Nosomma* as new genera closely allied to *Hyalomma* Koch and also proposed *Hyalommina* as a subgenus of *Hyalomma*. I agree with him in considering them to be separate genera, but I cannot regard his *Alloceraea* (proposed by him in 1918, pp. 62, 63) as a valid genus. He considers *Alloceraea* to be intermediate between *Haemaphysalis* Koch and *Aponomma* Neumann, and he proposed it for the *Aponomma*-like species *Haemaphysalis inermis* Birula. The differentiating characters of the genus *Alloceraea* as given by Schulze are "An *Aponomma* (bzw. *Amblyomma*) erinnern in beiden Geschlechtern die langen, im zweiten Glied nicht vorspringenden Palpen, das Fehlen des Haarfächers auf Glied zwei, der durch wenige Borsten ersetzt ist, und der Rüssel mit der an der Basis in Platten übergehenden Bezahnung, beim ♂ ferner der Verlauf der Genital und Analfurchen und das Stigma, an *Amblyomma* endlich die im Gegensatz zu Nuttall's Angaben auch nach innen scharf abgesetzten Randschildchen." These characters are, however, present in many other species of *Haemaphysalis*. The palps, according to Nuttall and Warburton (1915, p. 401), are long and have hardly any lateral salience in the female of *Haemaphysalis formosensis* Neumann. The paucity of hairs on palpal article II and the *Amblyomma*-like hypostome of *H. inermis* are apparently due to the peculiar blood-sucking habit (*vide* Nuttall and Warburton, 1915, pp. 545–7) of the species. The anal and genital grooves of the above type are found in most species of *Haemaphysalis*. The elongated spiracle of *H. inermis* to some extent approaches in shape that of *H. cornigera* var. *anomala* Warburton and certain other species of the genus. Finally, the scutes are distinct in *H. campanulata* Warburton. As stated by Warburton (1913, p. 130), "The genera *Haemaphysalis* and *Aponomma* have normally little in common, except the negative characteristics of the absence of eyes and anal plates"; but even the absence of eyes in these

two genera should not, in my opinion, be considered as evidence of relationship. In the genus *Haemaphysalis* it is a primitive feature, while in the genus *Aponomma* it appears to be a secondary characteristic. The genus *Aponomma* is closely related to the genus *Amblyomma* in which eyes are present, and the absence of eyes in *Aponomma* is probably due to the genus being parasitic on reptiles. The anterior portion of the body of the tick remains concealed under the scales of the reptiles and the eyes appear to have disappeared as a result of disuse. This is confirmed by certain species of *Amblyomma*, namely *A. sublaeve* Neumann and *A. helvolum* Koch, which are parasitic on scaly hosts and possess indistinct eyes (*vide* Robinson, 1926, pp. 245, 246 and 217, 218). In my opinion there is no relationship between the genera *Aponomma* and *Haemaphysalis* and the creation of a new genus, *Alloceraea* Schulze, for *Haemaphysalis inermis* Birula, which Nuttall and Warburton rightly referred to the genus *Haemaphysalis*, is quite unjustified and this name I relegated to the synonymy of *Haemaphysalis* (1928, p. 238).

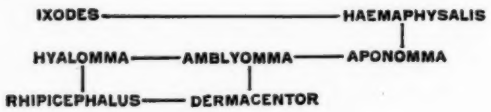

Fig. 1

I recognise only twelve valid genera of the family Ixodidae. They are *Ixodes* Latreille, *Haemaphysalis* Koch, *Rhipicephalus* Koch, *Boophilus* Curtice, *Margaropus* Karsch, *Rhipicentor* Nuttall and Warburton, *Dermacentor* Koch, *Hyalomma* Koch, *Amblyomma* Koch, *Aponomma* Neumann, *Nosomma* Schulze and *Cosmiomma* Schulze. I also consider *Hyalommina* Schulze and *Pterygodes* Neumann to be valid subgenera.

The family Ixodidae is a very compact family and its genera, though closely allied to one another, are difficult to place in their exact relationship, and no one has so far been able to decide with any degree of certainty which is the most primitive form among them. Neumann (1904, p. 447) was the first who attempted to ascertain the affinities of the genera by the study of the more important morphological characters. He published a scheme (see Fig. 1) of the relationship of the genera which were known to him at that time.

Subsequently, Neumann (1911, p. 8) arranged the genera under three tribes: (1) Ixodaria (=*Ixodes* Latreille further divided into subgenera *Ixodes*, *Ceratixodes* Neumann and *Eschatocephalus* Frauenfeld), (2) Rhipicephalaria (= *Rhipicephalus* Koch, *Margaropus* Karsch and *Hyalomma* Koch) and (3) Amblyommataria (=*Amblyomma* Koch, *Aponomma* Neumann, *Dermacentor* Koch and *Haemaphysalis* Koch). It is only in the last grouping of the genera that I differ from Neumann. *Haemaphysalis*, as I have discussed later, has no relationship with *Amblyomma* and should be considered, as pointed out by Banks (1908, p. 13) and Warburton (1907, pp. 89–91), as forming a separate group of its own.

Warburton's scheme is very useful for the purpose of *identification*, but it does not show the true relationship of the genera. This author has attached too much importance to the length of the rostrum and has consequently separated such closely related genera as *Hyalomma* from *Rhipicephalus* and *Dermacentor* from *Amblyomma*.

Jakob (1924, pp. 358–69) arranged the genera according to their natural affinities. His scheme (see Fig. 2) is also somewhat defective and does not show the real relationship amongst the genera. According to him *Rhipicephalus* is more closely related to *Amblyomma* than the latter is to *Hyalomma*, which is not

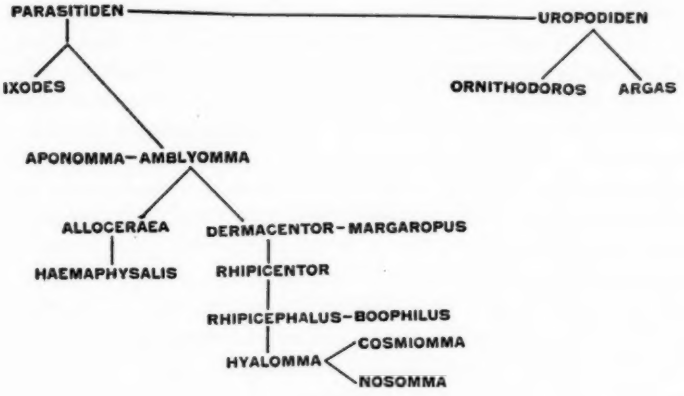

Fig. 2

borne out by our present knowledge of these genera. In attempting to justify his scheme, Jakob has laid special emphasis on minor characters which, as is shown hereafter, are of little systematic importance and are not even constant in different species of the same genus.

Besides the characters employed by previous workers for determining the relationship of the various genera I consider the setiferous ventral plate on palpal article I as being of some importance in this connection. The setiferous ventral plate on palpal article I is quite a separate structure in *Rhipicephalus*, *Rhipicentor*, *Nosomma* and *Hyalomma*, but is fused with palpal article I in *Amblyomma*, *Aponomma* and *Dermacentor* and is obsolescent in *Boophilus*, while it is totally absent in *Haemaphysalis* and *Ixodes*.

In determining the relationship of the various genera of the family Ixodidae I attach special importance to the ventral armature of the male, as represented by the ventral shields, plates or plaques. These secondary sexual characters are considered by some to be useless for the purpose of arranging the genera into higher groups, but I have found them very useful in connecting the genera with one another. These structures, viz. shields, plates and plaques, in my opinion represent different stages of development of the same structure in various

genera and probably indicate their line of evolution. In view of the comparatively close relationship of the various genera of this family with one another it is not desirable to combine them into any higher groups as Neumann has done. Like other workers on this group I have taken the genus *Ixodes* as the starting point, and have tried to connect the remaining genera with it.

The genus *Ixodes* according to Warburton, Banks and Neumann is distinct from the remaining genera of the family Ixodidae in having the anal grooves united in front of the anus. It is not very difficult to derive the condition of the anal grooves as found in the genera in which the anal grooves meet behind the anus from the one in the genus *Ixodes* when we know that in certain species of the genus *Ixodes*, as *I. rasus* Neumann and *I. holocyclus* Neumann, the anal grooves are united both in front of and behind the anus. The presence or absence of the festoons as pointed out by Jakob (1924, p. 360) is undoubtedly related to the development of the diverticula of the intestine, and their absence in the genus *Ixodes* is of no great importance as a differentiating character of the genus since they are also absent in *Margaropus* and *Boophilus*. I am, therefore, of opinion that these characters alone do not allow us to believe that the genus *Ixodes* is not related to other genera of the family Ixodidae, this belief being shared by Neumann (see Fig. 1).

If *Haemaphysalis* is related to any existing genera of Ixodidae it is to *Ixodes*, though this relationship is very remote. The ventral plates of the male which are present in *Ixodes* have totally disappeared in *Haemaphysalis*. Banks and Warburton have placed the latter genus in a separate group as it is not related to any other genus of Ixodidae. According to Schulze it is related more to the genus *Aponomma* than to *Amblyomma* through his new genus *Alloceraea*. Jakob, on the other hand, who believes in its direct relationship with *Amblyomma*, bases his view on the similarity of the bristle-formula of *H. inermis* Birula to that of the species of the genus *Amblyomma*. This bristle-formula, however, to which Jakob has attached so much importance, is, according to my observations, very variable in different species of the same genus[1], and in some cases even in individuals of the same species, and cannot, therefore, be of any great value for determining the relationship of the different genera. Jakob's (1924, p. 361) statement, "Ferner besitzen alle *Haemaphysalis* an jeder Coxa *einen* medianen kleinen Höcker oder (meist) nur an der 1. und 4. Coxa je einen langen, nach hinten gerichteten Dorn, den in gleicher Weise nur *Amblyomma*-Arten (*maculatum* Koch u.a.) noch besitzen," is very misleading and incorrect, as is clearly shown in Robinson's (1926, pp. 40–4) excellent figure

[1] For example, in the different species of *Hyalomma* there are two types of bristle-formulae. In *Hyalomma* (*Hyalomma*) *aegyptium* (Linnaeus) the bristle-formula is $\begin{matrix}2\\7\end{matrix}\Big|\begin{matrix}2\\7\end{matrix}$, which according to Jakob is the same as that of *Rhipicephalus*. In *Hyalomma* (*Hyalommina*) *kumari* Sharif it is $\begin{matrix}2\\4\end{matrix}\Big|\begin{matrix}2\\2\end{matrix}$, which is the same as that of *Amblyomma*, though the subgenus *Hyalommina* has no relation with *Amblyomma* and is more closely related to *Rhipicephalus*.

and description of *Amblyomma maculatum*, in which there are two spurs on coxa I, which is never the case in the genus *Haemaphysalis*. The coxal armature of *A. maculatum* is in no way similar to that of *Haemaphysalis*; for the remaining one or two common points which he gives between the two genera are of no importance and are found in some other genera as well. *Haemaphysalis* differs from *Amblyomma* in the absence of eyes, in having an inornate small body and in the dorsal retroverted spur on trochanter I. In my opinion there is no relationship between *Haemaphysalis* and *Amblyomma* or any other genus in which the anal grooves surround the anus posteriorly. It has the following important points in common with *Ixodes*, with which it has a far-fetched relation: absence of eyes, inornate body, dorsal retroverted spur on trochanter I which is present in *Ixodes apronophorus* Schulze (1924, pp. 281–4) and the genus *Haemaphysalis*, and the absence of the setiferous ventral plate on palpal article I.

The genus *Amblyomma* can easily be derived from *Ixodes* by the disappearance of the ventral plates in the male, the appearance of eyes, which are absent in *Ixodes*, and by the formation of the festoons. The five plaques which are present in the males of some species of *Amblyomma* (e.g. *Amblyomma longirostre* (Koch) and *A. geayi* Neum.) correspond to five plates present in male *Ixodes* behind the anus. The two outermost plaques may be considered as remnants of the epimeral plates, two internal to them as remnants of the adanal plates and the middle one as the remnant of the anal plate. The tendency towards the reduction in size of the ventral plates is observable in the epimeral and anal plates of *Ixodes acutitarsus* (Karsch) (*vide* Sharif, 1928, p. 234, Text-fig. 3 *a*). According to Jakob these two genera have sprung from a common stock and he believes in the closer relation of *Ixodes* with *Amblyomma* than with any other genus. This close relationship between these two genera he bases on the similarity of the capitulums of *Ixodes holocyclus* Neum. and *Amblyomma longirostre* (Koch). This similarity is, I think, of doubtful value, and in attempting to discover the true relationship between these genera it is also unwise to place too much emphasis on the form of the palps which vary a good deal in both. *Amblyomma* is related to *Ixodes*, but the relationship is rather remote and is practically of the same nature as that between *Hyalomma* and *Ixodes*.

The genus *Hyalomma* can easily be derived from *Ixodes* by the disappearance of the pregenital, median and anal plates and the anterior portion of the epimeral plates of male *Ixodes*, by the development of the remaining plates into prominent shields and also by the presence of eyes and the formation of festoons. According to Jakob, *Hyalomma* is the most distantly related genus to *Ixodes* and *Amblyomma* the most closely related to it. The only reason he (1924, p. 358) gives is that which he terms the "Palpenwinkel" "(den ebenfalls langpalpigen *Hyalomma*-Arten fehlt er!)." This palpal angle, for the discovery of which Jakob claims credit, was clearly recognised by Nuttall and Warburton (1911, p. 135) in the genus *Ixodes* as the following statement shows: "In most species the second article of the palp is narrowed at the base, leaving a space

between it and the hypostome, and this gives the ticks a peculiar facies easily recognized by the naked eye." This palpal angle (the space between the hypostome and the palp) is no doubt present in most species of the genus *Amblyomma*, but usually it is not so pronounced as in *Ixodes*. After careful examination of *Hyalomma* (*Hyalomma*) *aegyptium* (Linn.) I find that only a slight indication of it is present in the female but it is well indicated in the nymph of the same species. In my opinion the presence of this angle is not of such an important character as to help us in any way in determining the relationship of the genera. As I have pointed out before, *Hyalomma* is, in my opinion, as closely related to *Ixodes* as is *Amblyomma*, but the evolution of the genera has taken place along two different lines. There is a strong similarity between the coxal armatures of most species of *Hyalomma* and *Ixodes acutitarsus* (Karsch), which points to some natural affinity between the two genera. According to Jakob, *Hyalomma* is related to *Rhipicephalus*, which is related to *Dermacentor* through *Rhipicentor*, and *Dermacentor* is related to *Amblyomma*. This scheme of relationship between *Hyalomma* and *Amblyomma* is rather too indirect and a more direct connection between these genera does, I think, exist. If we exclude the ventral shields of male *Hyalomma* it becomes in all respects an *Amblyomma*. Furthermore, we have a similar tendency towards *Amblyomma* in the genus *Cosmiomma*, which is closely related to *Hyalomma*. As regards the capitulum and general appearance, *Amblyomma* resembles *Hyalomma* more than *Ixodes*, especially in the case of females which in these two genera are rather difficult to differentiate.

The close relationship between *Amblyomma* and *Dermacentor* has been fully realised by Banks and Jakob, but it is not in conformity with the scheme proposed by Warburton and I believe there is also a close relationship between *Hyalomma* and *Dermacentor*; my views on this point are based upon personal observations on the only species of the genus *Nosomma* which resembles *Dermacentor* in all respects except that it possesses ventral shields like those of *Hyalomma*.

The close relationship between *Boophilus* and *Rhipicephalus* has been emphasised by Neumann and many others. Neumann for many years included *Boophilus* in the genus *Rhipicephalus* and he considered the subgenus *Pterygodes* intermediate between the two.

Jakob has placed the genus *Margaropus* close to *Dermacentor*. This he does on account of its possessing large segments in the last pair of legs, in the absence of certain *Rhipicephalus* characters and on the similarity in the proportion of the palpal articles in the two genera. The common characters between *Margaropus* and *Dermacentor* that he gives I think are of minor importance and even of doubtful existence. He states that the basis capituli of *Margaropus* has no lateral salience; on the other hand Neumann (1907, p. 222) says of the base of the female of *M. lounsburyi* Neum. "la base plus de deux fois aussi large que longue, sub-hexagonale," and both his and Cooper and Robinson's (1907, p. 37, Text-fig. 4) diagrams of the female of the same species show that the lateral saliences on the base are present though not very prominent. The other point

in common according to Jakob[1] between the two genera is the presence of the dorsal retroverted spur on palpal article II; this is mentioned neither by Neumann nor by Cooper and Robinson in their accounts of *M. lounsburyi* and Jakob may have been misled regarding this point by Neumann's somewhat defective diagram of the female capitulum of *M. lounsburyi*. In my opinion the genus *Margaropus* has no intimate relation with the genus *Dermacentor*. According to Warburton the genus *Margaropus* is intermediate between *Rhipicephalus* and *Boophilus*; this view I am unable to subscribe to. The coxal armature, the general appearance of the capitulum, the absence of a well-developed setiferous ventral plate on the palpal article I, the simple hairs on the palps, the female scutum and the spiracle of the genus *Margaropus* point to a strong similarity with the genus

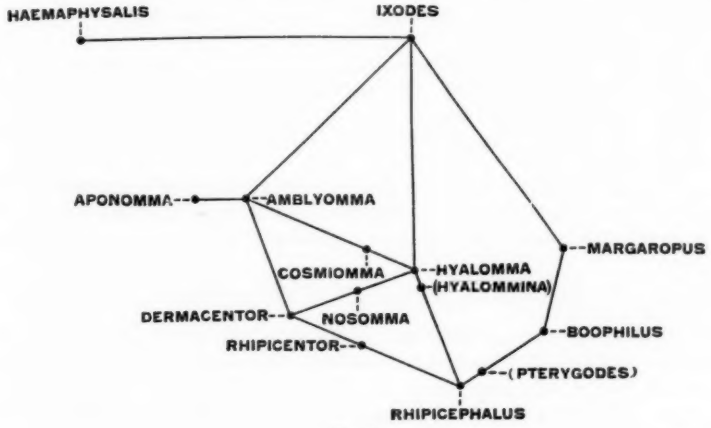

Fig. 3

Boophilus and are quite unlike those of the genus *Rhipicephalus*. In my opinion *Margaropus* is related to *Rhipicephalus* through *Boophilus*, but has no direct relation with it. The presence of a unique ventral shield in the male, the form of the spiracle and the absence of the festoons in the genus *Margaropus* show that it possesses affinities, though somewhat remote, with the genus *Ixodes*. The ventral shield in the male of *Margaropus* is, I believe, formed by the fusion of the adanal plates with the median plate, while the other plates which are present in the male of *Ixodes* have totally disappeared.

The genus *Aponomma* is closely related, as pointed out by previous workers, with the genus *Amblyomma*, from which it is differentiated by the absence of eyes and the broad body.

[1] Jakob's statement (p. 363), "Das 2. Glied trägt bei allen *Dermacentor*-Arten, bei *Rhipicentor* und *Margaropus* einen dorsalen, nach hinten gerichteten Anhang," is incorrect, as the dorsal retroverted spur is not present in many species of the genus *Dermacentor* and is also absent in the genus *Margaropus*.

Keeping in view the above-mentioned discussion, I propose the above scheme (Fig. 3) which shows the relationship of the existing genera of the family Ixodidae. The length of the connecting lines denotes approximately the degrees of affinity.

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ON THE BIOLOGY OF *ANAGRUS ATOMUS* (L.) HAL.: AN EGG PARASITE OF THE LEAF-HOPPER *ERYTHRONEURA PALLIDIFRONS* EDWARDS

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(With 11 Figures in the Text)

Introduction

In the spring of 1930, while examining primula leaves for eggs of the leaf-hopper, *Erythroneura pallidifrons* Edwards, it was noticed that some of these eggs were bright red instead of being as usual colourless and almost transparent. From these red eggs a small hymenopterous insect, one of the Mymaridae, was bred out; this insect has since been identified as *Anagrus atomus* (L.) Hal.

As early as 1833 Haliday described this species under its present name, but except that he states that it is common under trees in autumn he gives no indication of its habits. Abbé Pierre (1906) bred *Anagrus atomus* from the eggs of *Tettigonia viridis* L., one of the leaf-hoppers, but as the latter insect has only one generation in the year he is almost certain that the mymarid has some other host in addition to the jassid named above.

The first eggs of *Erythroneura pallidifrons* which were found to be parasitised by *Anagrus atomus* were on primulas which had been obtained from two localities: Wilmslow, Cheshire, and Fallowfield, Manchester; and shortly afterwards a few parasitised eggs were found on a species of *Salvia* in the glasshouses of the Manchester University Experimental Grounds at Fallowfield. After about a dozen adult mymarids had been obtained all traces of the parasite disappeared and, as attempts to make the insect breed in the laboratory had failed, nothing more could be done at that time.

During the next year no trace of the mymarid could be found, but in April 1932 numbers of *Erythroneura pallidifrons* eggs in the glasshouses at Fallowfield were found to be parasitised by *Anagrus atomus*. The parasitised eggs were found first on *Salvia* and later on *Mirabilis tubiflora*. In 1933 *Anagrus atomus* again appeared at Fallowfield, this time in the eggs of *Erythroneura pallidifrons* infesting a species of *Pelargonium*.

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Material and methods

Practically all the material used in the following account was collected from Fallowfield.

Owing to the extremely small size of *Anagrus atomus* it has been found very difficult to breed the insect in captivity. It was found, however, that the parasite would oviposit readily in leaf-hopper eggs in small pieces of leaf in corked tubes, but it was difficult to prevent the leaf from either shrivelling up or rotting before the insect could complete its development, so that only a few individuals were brought right through from the egg to the adult insect.

In 1930 and 1932 all the adult *Anagrus atomus* obtained were female, but Enock's collection of Mymaridae in the Manchester Museum contains four specimens of *Anagrus atomus*, three female and one male, and the description of the male in the following account is taken from this specimen. In April 1933 two male *Anagrus* were bred out and about a month later a third male also emerged.

Description

Haliday (1833) gives the following description of *Anagrus atomus*:

Gen. IV. *Anagrus.* Haliday.

Antennae maris 13-articulatae, feminae 9-articulatae, capitulo exannulato. Tarsi tetrameri. Abdomen sessile conico-acuminatum.

Caput valde oblatum vertice lineari fronte depressâ: antennae *feminae* articulo 3tio. brevissimo, sequentibus sub-equalibus ultimo majore oblongo; *maris* compressae crassiusculae articulis flagelli interioribus paulo brevioribus: thorax oblongus depressus, collari parvo attenuato, scutello brevi semiorbiculato; metathorace fere disjuncto, subitò depresso et dorso excavato: abdomen sessile metathoracis dorso incumbens, conicum elongatum, dorso deplanatum aut canaliculatum, ventre compressum carinatum, aculeo breviter exerto: pedes graciles: alae lineares plumato-ciliatae, anticae apice nonnihil dilatatae rotundatae: cilia haud ex ipso margine oriuntur sed interiùs unde margo alae pulcherrimè duplicatus S. striatus extat: nervus subcostalis tenuissimus.

Sp.l. *A. atomus* ♀. Pallido fuscoque varius alis hyalinus. (Long. 0·02; alar. 0·05.)

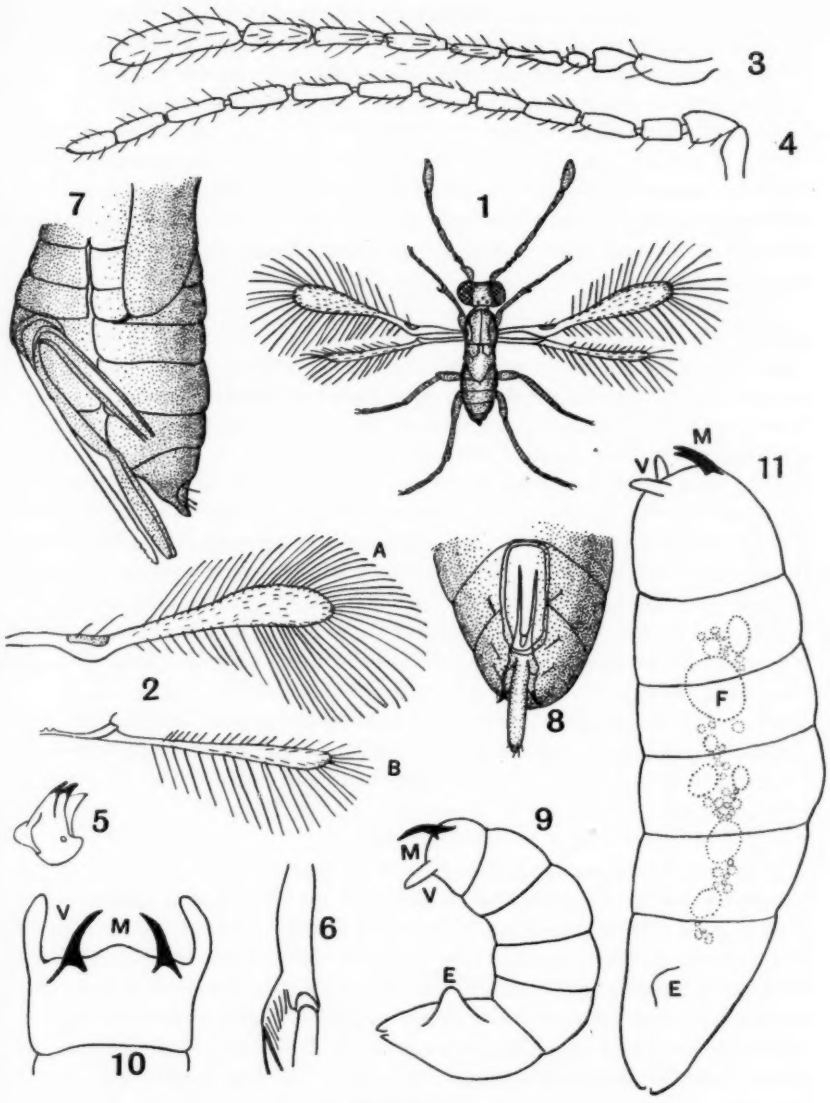
Ichneumon atomus Linn.

Caput, antennarum apex, prothorax et anus saepius subfusca; alae limpidae pulcherrimè ciliatae.

Upon grass under trees, in autumn: common.

The *Anagrus atomus* ♀ (Fig. 1) is approximately 0·6 mm. long; the forewing is also approximately 0·6 mm. long, so that the width of the insect with the wings extended is a little more than its total length; the eyes are black with a slight keel along the inner margin and the three ocelli are placed far back on the head; the antennae are rather shorter than the body, and the bases of the antennae are widely separated. In the female the antennae (Fig. 3) have nine segments, the scape is about twice as long as the pedicel and the first funicular segment is very short. The remaining segments tend to increase in size towards the apex, but the last segment is much larger than the others and forms a distinct club. The mandibles (Fig. 5) have three teeth.

The metathorax is prolonged backwards over the first segments of the



Anagrus atomus

Fig. 1. Female. ×36. Fig. 2. Wings: A, fore-wing; B, hind-wing. ×80. Fig. 3. Antenna. ♀. ×150. Fig. 4. Antenna. ♂. ×150. Fig. 5. Mandible. ×300. Fig. 6. Tibia of anterior leg. ×300. Fig. 7. ♀, abdomen showing ovipositor. ×150. Fig. 8. ♂ genitalia. ×150. Fig. 9. Early larva: *M*, mandible; *V*, ventral appendage; *E*, "ear-shaped" organ. ×150. Fig. 10. Head of early larva from the dorsal side: *M*, mandible; *V*, ventral appendage. ×150. Fig. 11. Late larva: *M*, mandible; *V*, ventral appendage; *F*, fat globules; *E*, remains of "ear-shaped" organ. ×150.

abdomen and bears a stout curved seta at its posterior end. The anterior tibiae have an apical plumose spur (Fig. 6). The fore-wings are distinctly bent so that the apex of the wing is directed towards the head, the marginal setae are very long on the distal half of the wing, they do not arise exactly from the wing margin but originate from a short distance on the inner side of it. The surface of the wing has a number of short setae evenly distributed except for a small bare area at the posterior side of the apex. The hind-wing is linear and petiolate with long strong setae on the posterior margin and shorter, less stout setae on the anterior edge. A double row of setae occurs on the surface of the wing (Fig. 2).

The abdomen is broadly sessile with the ovipositor emerging from the posterior end of the third segment on the ventral side (Fig. 7). The tip of the ovipositor is just visible beyond the apex of the abdomen. The ovipositor is composed of two straight blades slightly serrated at the tip; when not in use it is enclosed in a sheath, the two halves of which arise on the ventral side of the abdomen at the same place as the ovipositor itself.

The male insect is approximately the same size as the female. Except for the genitalia (Fig. 8) the most striking external difference between the two sexes is in the form of the antennae (Fig. 4). In the male the antenna has thirteen segments; the scape is only slightly longer than the pedicel, and the first segment of the funicle, instead of being markedly smaller than the others, is only slightly shorter and broader than the following segment. The terminal segments of the antenna are similar to each other except the last, which is pointed at the tip.

The adult *Anagrus* is an active insect, it can run rapidly over the leaves of the plant and is able to make short flights or jumps from leaf to leaf; it does not, however, seem to be capable of any sustained flight.

Oviposition

The *Anagrus atomus* ♀ appears to be fully mature as soon as she emerges from the pupal stage, and if suitable host eggs are available she begins to oviposit at once. The insect can reproduce parthenogenetically at any rate for the greater part of the year. For two years no males were obtained from the material collected, and though in 1933 three male insects were bred from leaf-hopper eggs, none of these were seen to copulate. When a piece of leaf containing *Erythroneura* eggs is put into a tube with an adult female *Anagrus*, the insect almost immediately begins to run about the leaf tapping the surface now and again with the antennae which are carried stretched out in front of her; when a leaf-hopper egg is encountered the parasite examines it several times, then raises the body slightly on the legs and slowly lowers the ovipositor until it is at right angles to the abdomen and thrusts it almost up to its base into the egg. *Anagrus atomus* takes about three minutes to deposit each egg, and one insect apparently laid nine eggs without any pause between each oviposition. More than one egg may be laid in each host egg. The parasite has

been seen to attack the same leaf-hopper egg five or six times, but although the eggs hatch and the larvae reach a certain stage in development, never more than one adult *Anagrus* has been bred from one egg of *Erythroneura pallidifrons*, though several adult *Anagrus urichi* have emerged from a single egg of the sugar-cane frog-hopper (Pickles, 1932). The largest number of larvae found in one leaf-hopper egg was three.

The *Anagrus atomus* ♀ lives only for a few days after emergence, and, though it has not been possible to estimate the number of eggs laid by one female, it is probable that the number of eggs laid is small as the percentage of parasitised eggs found on the plants was invariably low.

THE EGG

When the egg is dissected out of the ovaries of the insect it is seen to be an oval structure about 0·06 mm. in length and approximately three times as long as it is broad. At one end is a narrow stalk about half as long as the egg itself. In one leaf-hopper egg a structure which was probably the egg of *Anagrus* was found; this was an oval translucent body, approximately 0·12 × 0·06 mm., which was dissected out of an *Erythroneura* egg known to have been attacked by *Anagrus atomus*. The stalk of the egg seemed to have completely disappeared. Many other host eggs were examined without anything resembling the egg of the parasite being found.

EARLY LARVA (Fig. 9)

The young larva is extremely transparent and quite colourless except for the mandibles. There are six segments including the head, the terminal segment being much larger than the preceding ones. The head segment bears two large, curved, strongly chitinised mandibles, and ventral to these a pair of long, narrow unchitinised appendages (Fig. 10). From the ventral side of the sixth segment two short, stout papillae project; the anus is terminal. Ganin (1869), in describing a similar larva belonging to a species of *Polynema* and parasitic in the eggs of *Agrion*, gives it the name "Histriobdella-like" larva from its fancied resemblance to the worm of that name. He states that the ventral appendages of the head, which he calls antennae, although they cannot be homologous with true antennae, and the "ear-shaped organs" on the posterior segment are both hollow outgrowths from the body wall, and the cavity within these appendages is continuous with the body cavity of the larva. Many of the parasitic hymenopterous larvae possess more or less well-developed "sensory organs" on the head, which probably correspond with the "antennae" of *Polynema* and *Anagrus*, but the function of the "ear-shaped organs" is obscure.

There are appendages on the head, the very large mandibles being characteristic of the Protopod type of larva (Berlese, 1925), but there are no appendages in the thorax. The abdominal region is segmented, but here the large sixth segment may represent several segments not yet differentiated, as in *Phaenoserphus viator* Hal. Eastham (1929) describes a larva with a posterior

macro-segment which later divides and forms several segments. The Protopod type of larva represents a very low state of development and is generally found among insects in which the eggs are very small with little yolk.

Larvae of *Anagrus atomus* with well-developed appendages on the posterior segment have been found up to the seventh day after oviposition and may reach a size of 0·44 mm. Three larvae of this stage, all approximately the same size, have been found in one leaf-hopper egg.

Late larva (Fig. 11)

The full-grown larva is only slightly different from the preceding stage. The mandibles and the ventral appendages of the head are still conspicuous. The posterior appendages quite disappear by the time the larva is ready to pupate, but they become smaller gradually, and larvae of about 0·5 mm. may still have quite well-developed "ear-shaped organs." In the later stages the larva is often, but not invariably, bright red in colour, so that the parasitised leaf-hopper eggs are easily recognised in the leaf tissue. The body of the late larva contains a considerable amount of fat in the form of large globules. The full-grown larva is approximately 0·7 mm. in length and completely fills the host egg. In the description of *Anagrus bartheli* by Tüllgren (1916) the late larval stage where the "ear-shaped organs" have disappeared is the only one mentioned.

Pupa

The pupa is slightly smaller than the full-grown larva and lies inside the host egg with the body not curved at all. When the pupal stage is reached the bright red colour becomes darker and the colours of the adult insect are gradually assumed; the compound eyes, which are red at first, can be seen clearly through the chorion of the leaf-hopper egg and the leaf tissue in which it lies. When the adult *Anagrus* is ready to emerge, it cuts with the mandibles a neat round hole at one end of the egg and emerges head first on to the leaf. The wings expand very rapidly and are usually fully developed by the time the insect has withdrawn its legs and abdomen from the egg. It is possible that the larva spins a light cocoon inside the host egg as the leaf tissue round eggs which have contained the parasite does not collapse as easily as that round eggs from which the leaf-hopper itself has hatched.

Length of the life cycle

Anagrus atomus takes approximately sixteen days to develop from the egg to the adult insect. A few of the parasites were bred in an incubator at a temperature of 26–27° C.; the length of the life cycle in these insects varied from fourteen to eighteen days, but the majority took sixteen days to complete their development. This time agrees very well with the estimated length of the life cycle in the glasshouse during the summer months. In the incubator the *Anagrus* larvae were seven or eight days old before the host egg showed any

signs of being parasitised, that is, the length of the last larval and pupal stages together was approximately eight days, and in the glasshouse the adult parasite emerged seven to ten days after the egg had turned red.

SUMMARY

1. *Anagrus atomus* (L.) Hal. is a mymarid parasite of the eggs of *Erythroneura pallidifrons* Edwards.

2. The parasite is parthenogenetic, at any rate for the greater part of the year.

3. Two larval stages are described.

4. More than one egg may be laid in a single host egg and these eggs may hatch, but only one of the *Anagrus* larvae reaches maturity.

5. The development of *Anagrus atomus* takes about sixteen days.

6. The species is multi-voltine.

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A REVISION OF THE GENUS *NYCTERIBOSCA* SPEISER (DIPTERA PUPIPARA, STREBLIDAE)

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From the Wellcome Bureau of Scientific Research, London

(With 16 Figures in the Text)

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Introduction

The genus *Nycteribosca* was established by Speiser in 1900 for the species which were formerly placed in the genus *Raymondia*, *Strebla* or *Brachystarsina*. This author described one new species and redescribed four others which were already known. Quite recently the number of the species of this genus has been increased to nine by Falcoz. In this paper I have added seven new species, and also give a redescription and figures of all the known species. I have not included *N. diversa* Frauenfeld in my key, but at the end of this paper there is a description of this fly based on that of Frauenfeld (1856), and a copy of his drawing of the wing. The drawing shows very clearly the branch of the second longitudinal vein. *N. diversa* appears to be a very doubtful species; it has never been recorded since its original description was written. Moreover, according to Kessel (1925), there is no branching of the second longitudinal vein ($R\,2+3$.) in the family Streblidae.

The geographical distribution of the species is shown diagrammatically. There are nine species recorded from the Oriental region, and of these *N. gigantea* and *N. amboinensis* occur also in the Australian region. From the last-named regions we have on record six species in all. Three species occur in the Mediterranean parts of the Palaearctic region, and of these *N. africana* and *N. alluaudi* are also distributed in the Ethiopian region. It is seen from the diagram of the geographical distribution of the species of the genus that there is no record of the occurrence of the Palaearctic and the Ethiopian species in the Oriental region and *vice versa*. The distribution of the species and the names of their hosts are given at the end of the description of each species.

Diagram of geographical distribution of species of genus Nycteribosca

Mediterranean parts of Palaearctic region	*Nycteribosca africana* *Nycteribosca alluaudi* *Nycteribosca diversa*		Ethiopian region
Oriental region	*Nycteribosca gigantea* *Nycteribosca amboinensis* *Nycteribosca nigriceps* *Nycteribosca cucullata* *Nycteribosca falcozi* *Nycteribosca modesta* *Nycteribosca caudata* *Nycteribosca pygialis* *Nycteribosca speiseri*	*Nycteribosca surcoufi* *Nycteribosca pretiosa* *Nycteribosca rouxi* *Nycteribosca buxtoni*	Australian region

The figures illustrating this paper were made with the aid of a camera lucida from specimens mounted in Canada balsam. The wings, owing to their transparency, were mounted in the solution of cellulose nitrate of Preston (1930)[1], separately from the body, but on the same slide. This mounting medium gives very excellent results; the wings look almost as if they were mounted dry.

Finally, I wish to express my thanks to Dr H. Scott and Dr F. W. Edwards, of the British Museum (Natural History), for placing at my disposal the specimens of the genus *Nycteribosca* in the collection of that institution; to Mr R. W. Hayman, of the same institution, for checking my list of the bats and their geographical distribution; to Mr Kerrich, of the University Museum of Zoology, Cambridge, for the specimens from the collection of that Museum. My thanks are also due to Dr L. Falcoz, of the University of Lyon, who was very generous in sending me the type specimens of *N. rouxi* and *N. surcoufi* as well as of three undescribed new species. These three species are described here under the names already proposed by him.

[1] Cellulose nitrate (type Nobel's H × 2) 25, methyl ethyl ketone 50, triacetone 25, mixed together and stirred until dissolved. The specimens may be mounted either straight from absolute alcohol or from oil of cloves.

General morphology of the genus *Nycteribosca*

The species of the genus *Nycteribosca* are closely related in their structure to those of the genus *Raymondia*. However, they can be easily separated from them by several definable characters. The classification of the species of the genus in question is chiefly based on their morphology, which, with the exception of the head (Jobling, 1929), has been very little studied. This section is devoted to a brief description of the morphological characters of these flies.

The head is laterally compressed, broad in its anterior part and becomes narrow towards the occipital foramen, so that in nearly all the species of the genus it is cup- or funnel-shaped when examined from above. Its dorsal and ventral surfaces are convex. In *N. gigantea*, *N. nigriceps* and *N. cucullata* the head capsule is very darkly pigmented. Eyes are present; in the last-mentioned species they are very conspicuous and project laterally. The anterior part of the head is occupied by the fronto-clypeus, which bears the two-segmented antennae and is attached by the membrane to the genae laterally and to the vertex dorsally. The second segment of the antenna bears comparatively long arista, of which the distal thin part has a few branches. The vertex occupies the dorsal surface of the head and is subdivided into several anatomically distinct subregions (Fig. 2). The lateral setose areas are the laterovertices (*lv.*); anteriorly each of them passes into the gena and laterally, behind the eye, into the postgena. The narrow unpigmented, membranous strip, extending from the fronto-clypeus and between the laterovertices, is the mediovertex (*mv.*). The most posterior subregion of the vertex is the postvertex (*psv.*); this corresponds to the vertical triangle of other authors. This subregion is of different shape in the different species of this genus. The postvertex extends to the posterior border of the head, where it may be separated by a very fine streak from the occiput, or merged into the latter. The occiput occupies about half of the posterior surface of the head, extending ventrally to the occipital foramen. The regions lying ventrally to the occipital foramen and also occupying the ventral surface of the head are the postgenae; in the most anterior part of the ventral surface each of them merges into the gena. In the middle of the ventral surface of the head, the postgenae are separated from each other by the membrane; this membrane is stippled in all the figures showing the ventral aspect of the head. In the anterior part of this membrane is attached the labium, which is formed by the theca (*t.*) and the labella (*lab.*). In *N. gigantea* the labella form a very long, narrow, tubular structure, while in all the other species they represent a very small, short structure which consists of a cone-shaped basal part and a very short, narrow, anteriorly rounded distal part. Dorsally to the base of the labrum, which is enclosed in the labium, and below the fronto-clypeus, are attached the maxillary palps. They are very flat, oval in outline, and broadly or narrowly rounded in their anterior part; not obtuse as those of the genus *Raymondia*.

The thorax is almost spherical when viewed dorsally. Its dorsal surface is

convex, while the ventral is flat along the middle. The prescutum (mesoprescutum) is nearly of the same dimensions as the scutum (mesoscutum) from which it is separated by a very distinct and complete mesonotal suture. The scutellum (mesoscutellum) varies in the different species of the genus. It is triangular in *N. pretiosa*, in some species it is broadly rounded, while in others it is more or less crescent-shaped. In *N. rouxi* (Fig. 9) it is produced into a pointed process posteriorly, which is darkly pigmented and bent ventrally. The humeral processes are absent in this genus. The largest part of the ventral surface of the thorax is occupied by the sternopleurae (*strn.*). On each side of the thorax each sternopleura merges into the mesopleura (*mes.*) and the pteropleura (*pter.*). The latter sclerites are separated from each other by the mesopleural suture (*m.s.*). Above the middle coxa lies the hypopleura and above this the metapleura (*met.*). In many species of this genus each metapleura is produced into a conical process pointed posteriorly (Figs. 14 B and 15 F). Behind the middle coxa each hypopleura merges into the pleurotrochantin (*pleu.*). The pleurotrochants occupy the posterior part of the ventral surface of the thorax. They are separated from the sternopleurae by the suture, while their posterior border merges into furcasternae, which, as those of the genus *Raymondia*, are crowded posteriorly, forming only the posterior edge of the ventral surface of the thorax.

The legs are strong, the hind pair is slightly elongated and the last tarsal segment is considerably enlarged. In *N. gigantea*, *N. nigriceps* and *N. modesta* the front coxae are very widely separated, in the rest of the species the front coxae are more or less close together.

The wings (Fig. 2 C) are elongated, not broad as those of *Raymondia*. They have five longitudinal and two cross-veins. The costal vein (*c.*) extends along the wing margin as far as the fourth longitudinal vein (*M* 1+2.), it is very setose in all the species; the setae are especially very long on the anterior margin. The subcostal vein is absent, but the humeral vein (*h.*) is present. The first longitudinal vein (*R* 1.) is the strongest; together with the costal vein it encloses the costal cell. In the basal part of the wing this cell is separated by the humeral vein from the humeral cell. The second longitudinal vein (*R* 2+3.) separates the marginal cell (1 *R.*) from the submarginal (3 *R.*). This vein affords a useful character for the separation of the species; in some of them its distal part is sharply bent towards the costal margin of the wing (Figs. 2 C, 3 B, 14 A and 15 A). The very short part of the third longitudinal vein (*R* 4+5.) is pale; together with the anterior cross-vein (*r-m.*) it separates the first basal cell from the submarginal (3 *R.*) and the first posterior cell (5 *R.*). The fourth longitudinal vein (*M* 1+2.) affords a very useful character; the length of the basal part of this vein with regard to its part between the anterior and the posterior cross-veins differs very much in some species. The posterior cross-vein (*M* 3+4.) separates the second posterior cell (2 *M* 2.) from the discal cell (1 *M* 2.), which is confluent with the second basal cell. The fifth longitudinal vein (*M* 4+*Cu* 1.) does not reach the wing margin; the distance between the

end of this vein and the posterior margin of the wing may vary even in the same species. This vein, together with the posterior margin of the wing, encloses the third posterior cell (*Cu* 1.). The posterior margin of the wing, from the fourth longitudinal vein, is fringed with very thin bristles. In this genus there are present two anal veins, the first of which (1 *A.*), together with the basal part of the fifth longitudinal vein, encloses the anal cell (*A.*), while the second vein (2 *A.*) separates the alula (*al.*) from the basal part of the wing. The alula is much better developed in this genus than in *Raymondia*, and its posterior margin is fringed with moderately long but very thin bristles. Arising from the posterior part of the base of the wing is the calipteron (*ca.*). This structure represents the modified and sclerotised squama. The halteres are moderately large, and their knob-like distal part bears a few very tiny bristles.

The abdomen is sacciform; its shape, however, in these flies, and especially in the female, is largely determined by the size of the larva developing in the uterus and the amount of blood taken during feeding. The abdomen of the female ends in an apical cone, differing in shape in the different species. The cone is attached by a membrane to another part of the abdomen, which is formed by one ventral and two lateral plates (*pl.*, Figs. 5 and 6). The lateral plates apparently represent the modified tergum and the ventral one the sternum of one of the posterior abdominal segments. They are better seen in the unfed flies, in which they are separated from the rest of the abdomen by a constriction of the membrane (Fig. 6 C). The basal tergite-like part of the abdomen forms a large lobe on each side of the dorsal surface. These lobes bear many strong, long setae, those arising from the posterior margin of these parts being the strongest abdominal setae. In the extreme antero-lateral part of the abdomen on each side of the narrow base there is another lobe-like elevation; it is very small and bears a few short setae. The dorsum of the abdomen has two longitudinal rows of very strong setae directed postero-laterally, and laterally to these several short but strong setae. The surface of the dorsum between the longitudinal rows of the strongest setae is naked and is depressed; it forms a longitudinal groove in which lie the folded wings at rest. The ventral surface of the abdomen is beset with very small setae, but these gradually become longer in the lateral parts of the abdomen. In *N. pretiosa* and *N. modesta* the setae of the ventral surface gradually become much stronger in the posterior part of the abdomen, where they very much resemble spines (Figs. 5 C and 8 B). The basal sternite-like portion of the abdomen is slightly sclerotised; it is triangular in outline and is beset with slightly stronger setae than the rest of the ventral surface. In *N. gigantea* the ventral surface of the abdomen shows traces of segmentation (Fig. 2 B).

The abdomen of the male differs from that of the female in the following respects: It ends in a large sclerotised, globular or conical hypopygium (*hyp.*). In the ventral surface of the hypopygium there is a median slit-like opening, through which the aedeagus (*æd.*) may protrude. Near the anterior edge of this opening there are present two digitiform structures (*dig.*), each carrying

two setae. A pair of digitiform or globular structures is also present near the posterior edge of the opening, these are provided with one or two setae, while on each side of the genital opening is attached the clasper (*cl.*), which is provided with small denticles along the outer edge near its distal end. Owing to the limited material at my disposal I was unable to study properly the internal parts of the genitalia, therefore the description given here is very incomplete. As in the genus *Raymondia* there are present two long apodemes inside the abdomen; in some species one of these is distinctly longer than the other. To the posterior end of the longest apodeme is attached the aedeagus, while the posterior end of the other apodeme is broadened, forming a short gutter through which the aedeagus passes during its eversion. Above or slightly lateral to the guttered part there is seen a bow-shaped rod, but it is not distinct in all the species of this genus.

It has already been mentioned at the beginning of this section that the species of the genus *Nycteribosca* are closely related in their structure to those of the other genus of the old world, *Raymondia*, which was previously studied by me (Jobling, 1930). During the present study some new generic characters have been found. It will therefore be useful to add these characters to those already known and include in this paper the description of these two genera.

Genus **Raymondia** Frauenfeld, 1855

Head rounded laterally, as broad as the anterior part of thorax, or more or less quadrangular and narrower than the thorax, dorso-ventrally flattened, closely articulated to the thorax, with concavity on each side of occipital foramen for the front coxae. Eyes absent; postvertex if distinct, very narrow and broad; arista of antenna short and thick with many thin branches; palps nearly as long as broad, obtuse. Thorax somewhat flattened; mesonotal suture not well defined or incomplete in the middle; humeral processes present. Wings twice as long as broad, with five longitudinal veins; first longitudinal vein very much thicker than the second vein, with setae along its entire length; first anal vein and anal cell absent; very few setae, or none in humeral and costal cells.

Genus **Nycteribosca** Speiser, 1900

Head broad in its anterior part, becoming narrow towards occipital foramen, its dorsal and ventral surfaces convex; no depressions for the front coxae; subregions of the vertex distinct; arista of antennae flagelliform with a few branches in its distal half. One-facetted eyes present; distal end of palps rounded. Thorax almost spherical, with very distinct, complete mesonotal suture; humeral processes absent. Wing more than twice as long as broad, with five longitudinal veins; first longitudinal vein not much thicker than the second, with setae in its distal part only; first anal vein and anal cell present; numerous setae in humeral and costal cells, those near costal margin and in humeral cell distinctly longer.

KEY TO THE SPECIES OF THE GENUS *NYCTERIBOSCA*

1. Head dark, *i.e.* distinctly more pigmented than the body; eyes very conspicuous, projecting laterally 2
   Head not darker than the body; eyes neither very conspicuous nor projecting laterally 4

2. Postvertex ovoid or oval; flies more than 3 mm. long 3
   Postvertex large, triangular; flies less than 2 mm. long (Fig. 1) **N. cucullata** sp.n.

3. Labella (*lab.*) as long as or a little longer than theca; greater part of prescutum and anterior half of scutum bare (Fig. 2) ... ... **N. gigantea** Speiser.
   Labella very much shorter than theca; prescutum with many small setae (Fig. 3) **N. nigriceps** sp.n.

4. Third and fourth longitudinal veins divergent near wing margin 5
   Third and fourth longitudinal veins parallel near wing margin 12

5. Basal pale part of third longitudinal vein as long as or shorter than the anterior cross-vein; second longitudinal vein gently bent towards costal margin 6
   Basal pale part of third longitudinal vein longer than the anterior cross-vein; second longitudinal vein strongly bent towards costal margin (Fig. 4) ... **N. falcozi** sp.n.

6. Thorax as long as or a little longer than broad[1] 7
   Thorax broader than long 9

7. Postvertex piriform; scutellum rounded posteriorly; very setose flies 8
   Postvertex ovoid; scutellum triangular, pointed posteriorly (Fig. 5) **N. pretiosa** Falcoz.

8. Theca conical, as long as broad at the base; alula rounded (Fig. 6) **N. amboinensis** Rondani.
   Theca rounded laterally, slightly broader than long; alula pointed (Fig. 7) **N. surcoufi** Falcoz.

9. Basal part of fourth longitudinal vein as long as or a little shorter than the part of this vein between anterior and posterior cross-vein; wings 2·5–2·7 mm. long 10
   Basal part of fourth longitudinal vein distinctly longer than the part of this vein between the anterior and posterior cross-vein; wing 2 mm. long (Fig. 8) **N. modesta** sp.n.

10. Scutellum broadly rounded, or slightly pointed without process 11
    Scutellum produced into a pointed process posteriorly, this is darkly pigmented and bent ventrally (Fig. 9) ... ... **N. rouxi** Falcoz.

11. Postvertex ovoid, its anterior end narrower than the posterior; scutellum broadly rounded (Fig. 10) ... ... **N. africana** Walker.
    Anterior end of postvertex broadly rounded, posterior end pointed; scutellum triangular, slightly pointed (Fig. 11) ... ... **N. buxtoni** Falcoz.

12. First longitudinal vein not parallel to the costal vein; distal part of second longitudinal vein strongly bent towards costal margin; metapleura (*met.*) with conical process... 13
    Middle part of first longitudinal vein almost parallel to the costal vein; distal part of second longitudinal vein gently bent towards costal margin; metapleura without conical process (Fig. 12) ... ... **N. speiseri** sp.n.

[1] The length is taken between the anterior border of the thorax and the posterior border of the scutellum, the breadth across the middle of the thorax.

13. Postvertex triangular; eyes small; basal part of third longitudinal vein as long as or shorter than the anterior cross-vein 14
Postvertex rounded, very distinct; eyes large; basal part of third longitudinal vein longer than the anterior cross-vein (Fig. 13) ... ... **N. pygialis** sp.n.

14. Theca large, conical, broad at the base; basal pale part of third longitudinal vein much shorter than the anterior cross-vein; scutellum slightly triangular; wings 3 mm. long (Fig. 14) ... ... **N. alluaudi** Falcoz.
Theca ovoid; basal pale part of the third longitudinal vein as long as the anterior cross-vein; scutellum crescent-shaped; wings 2·5 mm. long (Fig. 15) **N. caudata** sp.n.

Nycteribosca cucullata sp.n.

(Fig. 1)

One type specimen, ♂, from the collection of the Cambridge Museum. This is a very distinct species; it resembles *N. gigantea* and *N. nigriceps* in its darkly pigmented head, but can be easily separated from them by its small size, very broad mediovertex and the large triangular postvertex.

Body 1·7 mm. long (on slide). Head dark, much more pigmented than the body; eyes distinct, projecting laterally; mediovertex broad, very distinct; postvertex large, triangular; lateravertices with moderately strong setae; occiput bare behind the postvertex. Theca of the labium rounded, nearly as long as broad; labella very small. Small setae arise from the inner border of each postgena, behind them there is a second row of slightly longer setae. Palps nearly as long as broad.

Thorax spherical when viewed from above; prescutum moderately covered with setae, those arising from its anterior part are longer; scutum with two long setae arising from the middle and a few from its lateral margins, otherwise its surface sparsely covered with small setae; scutellum very broadly rounded, with four very long setae arising from the anterior half of its surface and several small setae close to its posterior edge; ventral surface of the thorax densely covered with very small setae.

Wings 1·9 mm. long; distal part of second longitudinal vein more or less sharply bent towards costal margin; third and fourth longitudinal veins almost parallel near the apex of the wing; setae of the costal and the marginal cells crowded near the costal margin. Legs long, femora with a few strong and many small setae; tibiae with numerous very small setae, posterior surface of the hind tibiae with longer setae.

Abdomen with a few strong, long setae in the longitudinal rows of its dorsal surface, and two strong setae in each postero-lateral part; its ventral surface densely covered with very small setae. Hypopygium semispherical, sclerotised, and occupying ventral position; its lateral surface bears many setae, these are small in the anterior part and become gradually much longer towards the posterior part. On each side of the base of the hypopygium, but separated

from it by a membranous space, there is a sclerotised, narrow, curved plate, its surface covered with setae which have the same disposition as those of the lateral surface of the hypopygium. Apodemes of the genitalia rod-shaped with bent distal ends, that of the aedeagus twice as long as the other.

From *Taphozous melanopogon* Temm., Matugama Estate, Kalutara, Ceylon, 100 ft., collected by Captain W. W. A. Phillips.

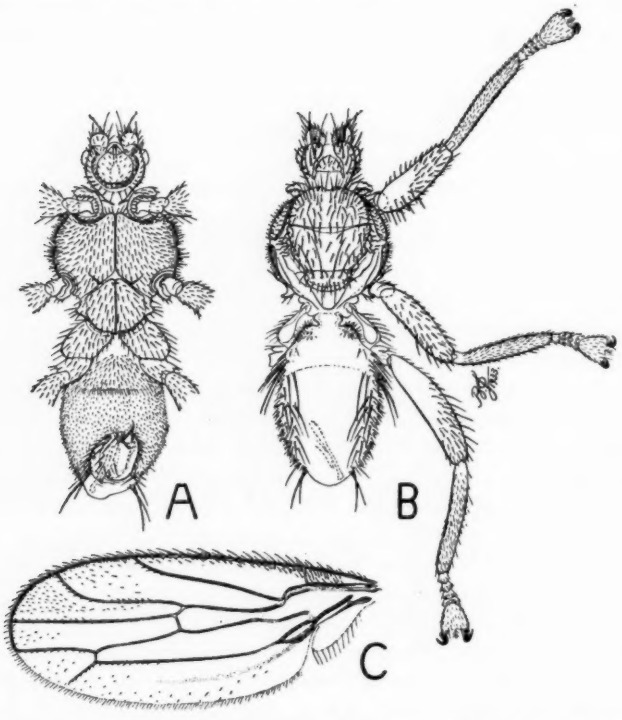

Fig. 1. *Nycteribosca cucullata* sp.n. A, ventral, and B, dorsal views of male; C, wing.

Nycteribosca gigantea Speiser, 1900

(Fig. 2)

This species has already been very well figured by Ferris (1924*a*), but for the sake of completeness I include the drawings of the male, the only specimen studied by me.

Body 3·5 mm. long (on slide), 4–4·5 mm. according to Ferris. It is the largest species of the genus; it resembles *N. nigriceps*, from which, however, it can be separated by its long labella (*lab.*), the chaetotaxy of the prescutum and the scutum, and the presence of traces of segmentation on the ventral surface of the abdomen.

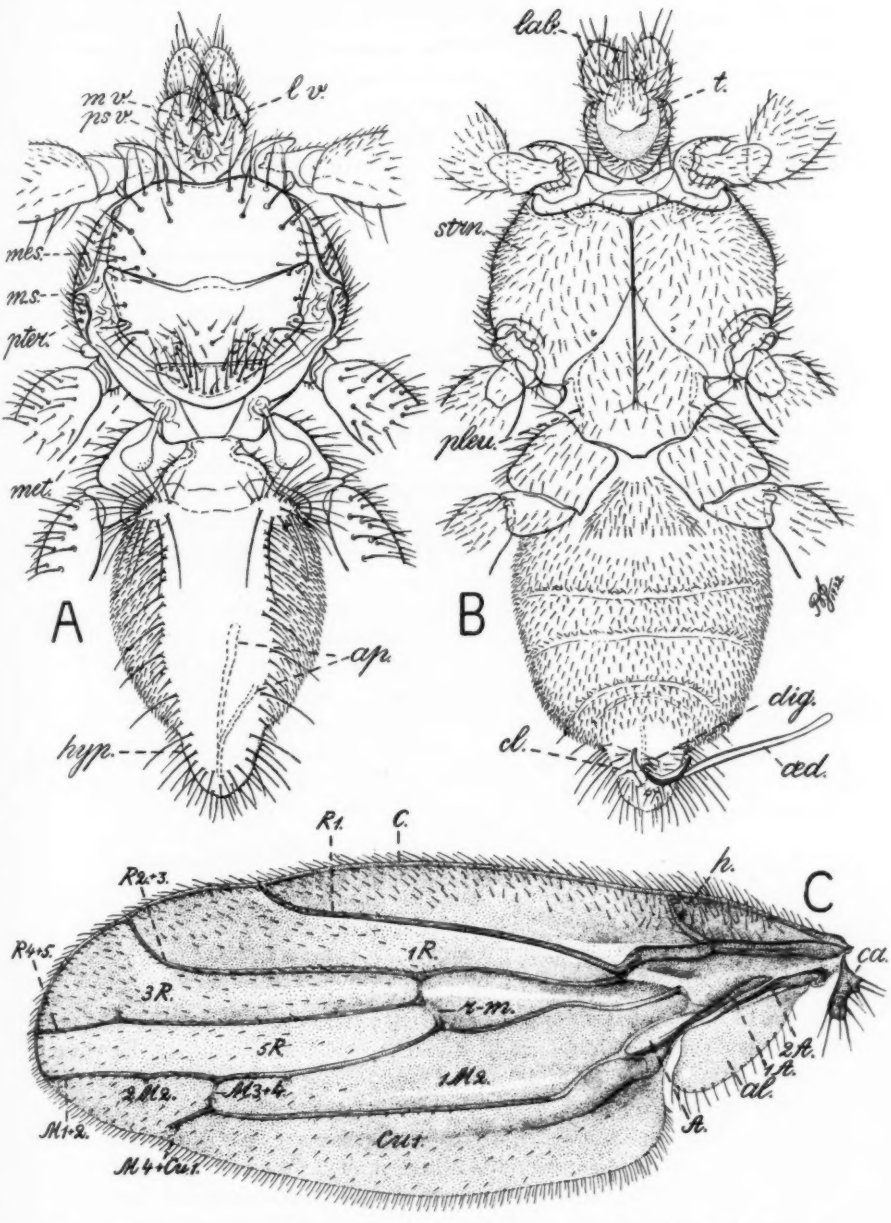

Fig. 2. *Nycteribosca gigantea* Speiser. A, dorsal, and B, ventral views of body of male; C, wing.

Head dark, much more pigmented than the body; eyes large, projecting laterally; lateravertices with moderately strong and small setae in addition to three long, strong setae arising from the anterior part; mediovertex narrow; postvertex ovoid with a few very small setae; a few small setae arise from the margin of the occiput, behind the postvertex. Palps distinctly longer than broad. Labella (*lab.*) as long as or a little longer than the theca (*t.*). Inner margin of each postgena fringed with setae; lateral parts of the postgenae bear rather long but thin setae.

Thorax somewhat rounded when viewed from above; prescutum with a few long setae in its anterior part and a few shorter ones near the lateral margins; the scutum bears a few setae near the lateral margins and a small group of erect setae of the same length in its posterior part; scutellum broadly rounded with many long and a very few small, erect setae. The ventral surface of the thorax bears many small setae. Dorsal part of mesopleurae and pteropleurae with stout setae. Legs strong; front coxae widely separated; dorsal side of femora with strong, long setae, ventral with small setae; tibiae more thickly beset with small setae.

Wings 3·9 mm. long. Second longitudinal vein strongly bent towards costal margin in its distal part; third and fourth longitudinal veins parallel near the apex of the wing; first basal cell enlarged in its anterior part; fifth longitudinal vein abruptly bent in its basal part.

Abdomen with a few long setae in the dorsal rows; its ventral surface bears many small setae and shows traces of segmentation. Hypopygium (*hyp.*) longer than broad, its distal end more or less rounded; it has many long setae on the lateral surfaces and a few setae on the posterior part of the dorsal surface, the ventral surface bears much smaller setae.

This species has been recorded from Gumba Islands, Bismarck Archipelago and the Philippine Islands, from two species of bats of the same genus: *Dobsonia sumbana* K.And. and *D. peroni* Geoffroy.

Nycteribosca nigriceps sp.n.

(Fig. 3)

One type specimen, ♂, from the collection of the British Museum (Natural History). Resembles *N. gigantea*, but can be easily separated from it by its very small labella, the presence of many very small setae on the prescutum and the absence of traces of segmentation on the ventral surface of the abdomen.

Body 3·2 mm. long (on slide). Head dark, distinctly more pigmented than the body; the lateravertices, besides three long setae arising from their anterior part, bear many small setae; mediovertex very narrow; postvertex oval with many small setae, a few small setae behind it on the occiput; eyes large, projecting laterally; the lateral and posterior surfaces of the postgenae bear many small setae. Labella form a small conical structure which is very much shorter than the theca (*t.*). Palps slightly longer than broad.

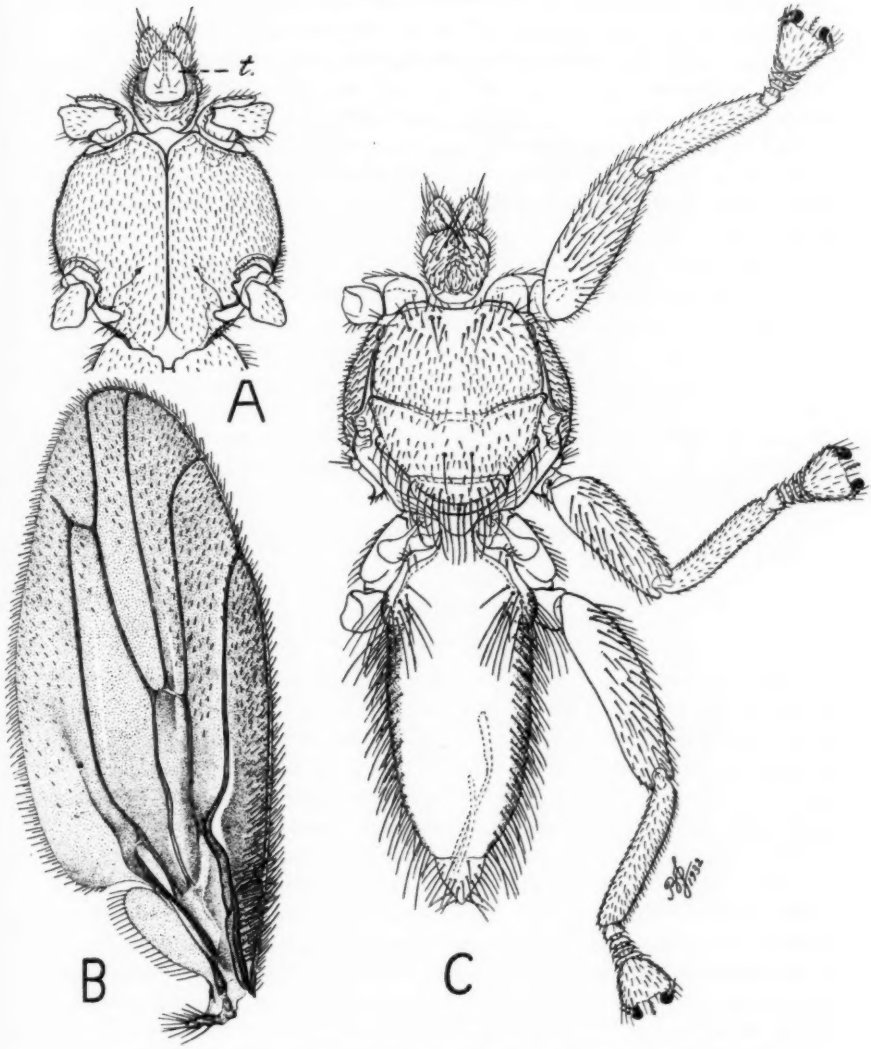

Fig. 3. *Nycteribosca nigriceps* sp.n. A, ventral view of head and thorax; B, wing; C, dorsal view of male.

Thorax rather darkly pigmented; prescutum larger than scutum, it bears a few long setae in the anterior part, the rest of its surface has many very small setae; scutum with a row of very small setae near the mesonotal suture and with setae of the same size in its lateral and the posterior parts; two long, thin setae arise from the middle of its posterior part and a few from the lateral borders; scutellum very broadly rounded with many long setae and a few small ones. Ventral surface of the thorax beset with numerous very small setae; mesopleurae and pteropleurae with many long setae in their dorsal part. Legs rather long; front coxae widely separated; the dorsal surface of femora with many strong setae, the ventral surface of femora and all the tibiae beset with small setae.

Wings 3 mm. long; distal part of the second longitudinal vein sharply bent towards costal margin; third and fourth longitudinal veins slightly divergent near the apex of the wing; first basal cell not enlarged in its anterior part; fifth longitudinal vein very gently bent in its basal part; costal and marginal cells with very many setae, those in the basal part of the costal and in the humeral cells are much longer.

Abdomen with many long, strong setae in the two dorsal rows; its ventral surface bears numerous very small setae. Hypopygium conical, with many long setae arising from its dorsal and lateral parts. Apodemes of the genitalia long, with their distal parts spatulated; digitiform structures globular.

From unknown species of bat, Bidi caves, Borneo.

Nycteribosca falcozi sp.n.

(Fig. 4)

One type specimen, ♂, from the collection of the British Museum (Natural History). It differs from *N. cucullata*, *N. gigantea* and *N. nigriceps* by its pale head. From all the other species which have the third and the fourth longitudinal veins divergent near the wing margin, it can be easily separated by the sharp curvature of the distal part of the second longitudinal vein, by the pale basal part of the third longitudinal vein, which is distinctly longer than the anterior cross-vein, and by its small size and very pale colour.

Body 2 mm. long (on slide). Head of the same colour as the body; eyes small, not very distinct; lateravertices with long thin setae; mediovertex very narrow, not quite distinct; postvertex ovoid, with a few small setae; a few small setae on the occiput, behind the postvertex; postgenae beset with moderately long but thin setae; palps longer than broad, rounded anteriorly. Theca as long as broad, rounded.

Thorax spherical when examined from above; prescutum and scutum beset with many long, thin setae; scutellum broadly rounded; from its surface arise many very long, thin setae; sternopleurae with many very small setae; pleurotrochantines more setose than sternopleurae; each metapleura with conical process. Dorsal surface of femora with long setae, ventral surface with many very small setae; tibiae densely beset with very small setae.

Wings 1·9 mm. long; second longitudinal vein sharply bent towards costal margin in its distal part; basal pale part of the third longitudinal vein distinctly longer than the anterior cross-vein; humeral cell with long setae; setae of the costal and the marginal cells crowded near the costal vein, the rest of the wing surface sparsely covered with setae; alula very broadly rounded.

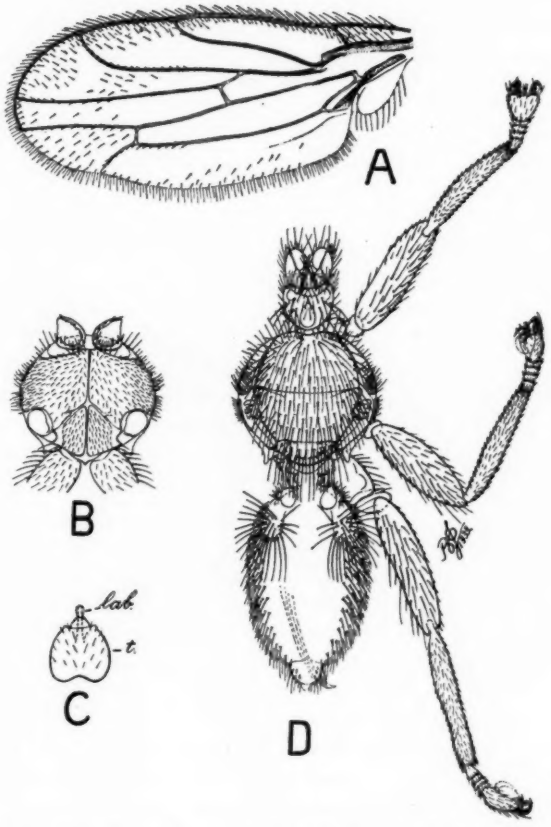

Fig. 4. *Nycteribosca falcozi* sp.n. A, wing; B, ventral view of thorax; C, ventral view of labella (*lab.*) and theca (*t.*); D, dorsal view of male.

Abdomen with many long setae in the two longitudinal rows of its dorsal surface, laterally to these much smaller setae, which gradually merge into very small and far more numerous setae of the ventral surface of the abdomen. Hypopygium spherical, with a very small, conical posterior part; its lateral surfaces bear moderately long setae, the ventral surface has very small setae on each side of the genital opening.

The true host of this species is not known; on the label is written: "From

Rhinolophus malayanus Bonhote, or *Hipposideros larvatus* Horsfield, Biserat, Jalor, Malay Peninsula."

Nycteribosca pretiosa Falcoz, 1924

(Fig. 5)

Three female specimens were examined from the collection of the British Museum (Natural History). Judging by the general structure, this species may be put between *N. africana* and *N. amboinensis*. It is more setose than the

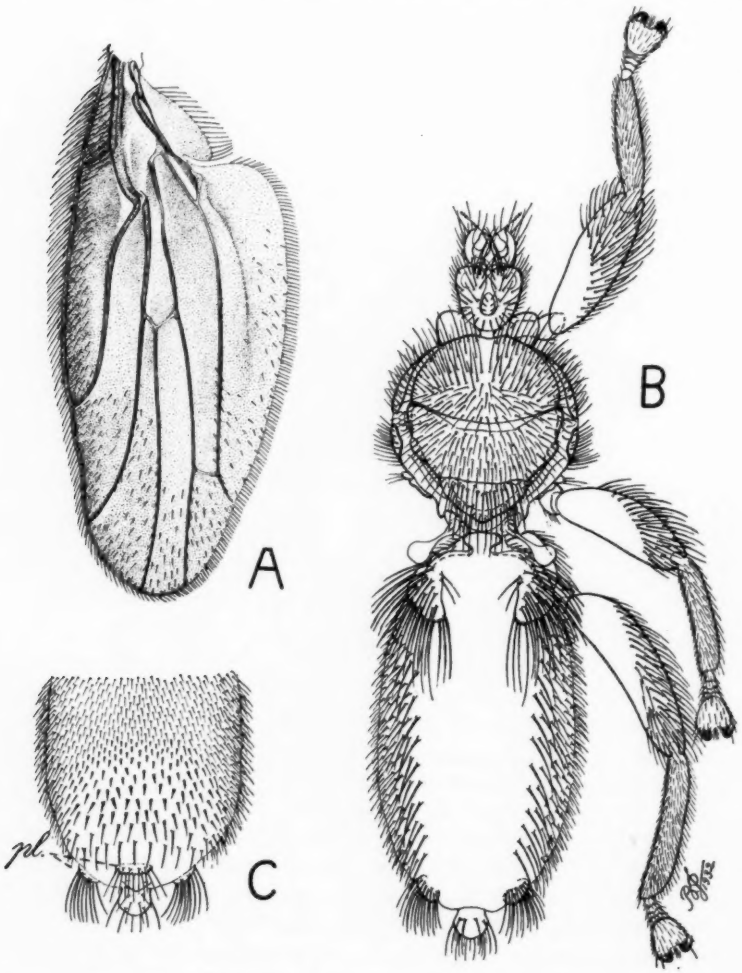

Fig. 5. *Nycteribosca pretiosa* Falcoz. A, wing; B, dorsal view of female; C, ventral view of posterior end of abdomen of same.

former and less than the latter. From both of them it can be easily separated by its distinctly triangular scutellum.

Body 3–3·1 mm. long (on slide). Head not darker than the body; each laterovertex has, besides three long setae arising from the anterior part, many much smaller setae; mediovertex not quite distinct, narrow; postvertex ovoid with small setae; a few small setae behind the postvertex on the occiput; eyes small; postgenae very setose. Palp longer than broad. Theca of the labium more or less conical; labella very small.

Thorax spherical, as long as broad when examined from above. Prescutum and scutum densely covered with long setae; the dorsal part of the mesopleurae and pteropleurae densely beset with slightly shorter setae; scutellum distinctly triangular, with many very long and a few short setae. The ventral surface of the thorax has numerous very small setae. Each metapleura has a small knob-like process projecting posteriorly. Legs stout; the dorsal surface of femora densely beset with very long setae, the ventral with very small setae; each tibia beset with many small setae, which are much longer on the dorsal surface of the hind tibiae; the ventral surface of coxae and trochanters of the hind legs densely covered with small setae.

Wings 2·6 mm. long. Second longitudinal vein very gently bent towards costal margin in its distal part; third and fourth longitudinal veins divergent near the apex of the wing; the distal end of alula broadly rounded; the humeral, costal and marginal cells are the most setose; the setae near the costal margin are very long in the basal part of the wing and gradually become shorter towards the apex.

Abdomen with very many long setae on each side of the bare part of its dorsal surface; towards the ventral surface of the abdomen the setae become gradually smaller. Ventral surface beset with very many small setae which in the posterior part of this surface become very much stouter and resemble spines (Fig. 5 C). Lateral plates of the abdomen of the female distinct, with very many long setae; the posterior cone of the abdomen small, as long as broad, rounded posteriorly, with four dorsal, two lateral and two ventral moderately long setae, and a few small setae arise from its ventral surface. Basal tergite-like parts with many long, strong setae.

This species has only been recorded from the New Hebrides, from *Miniopterus australis* Tomes.

Nycteribosca amboinensis (Rondani, 1878) Speiser, 1900

Brachytarsina amboinensis Rondani, 1878

(Fig. 6)

Several specimens were examined from my own collection and from the collection of the British Museum (Natural History). This species has already been figured by Ferris (1924*b*). It very much resembles *N. surcoufi*, from which it can be distinguished by the characters shown in the key.

Body 3–3·2 mm. long (on slide). Head not darker than the body, very convex dorsally; each laterovertex has, besides the three long setae of its anterior part, many slightly shorter setae; mediovertex narrow, not very

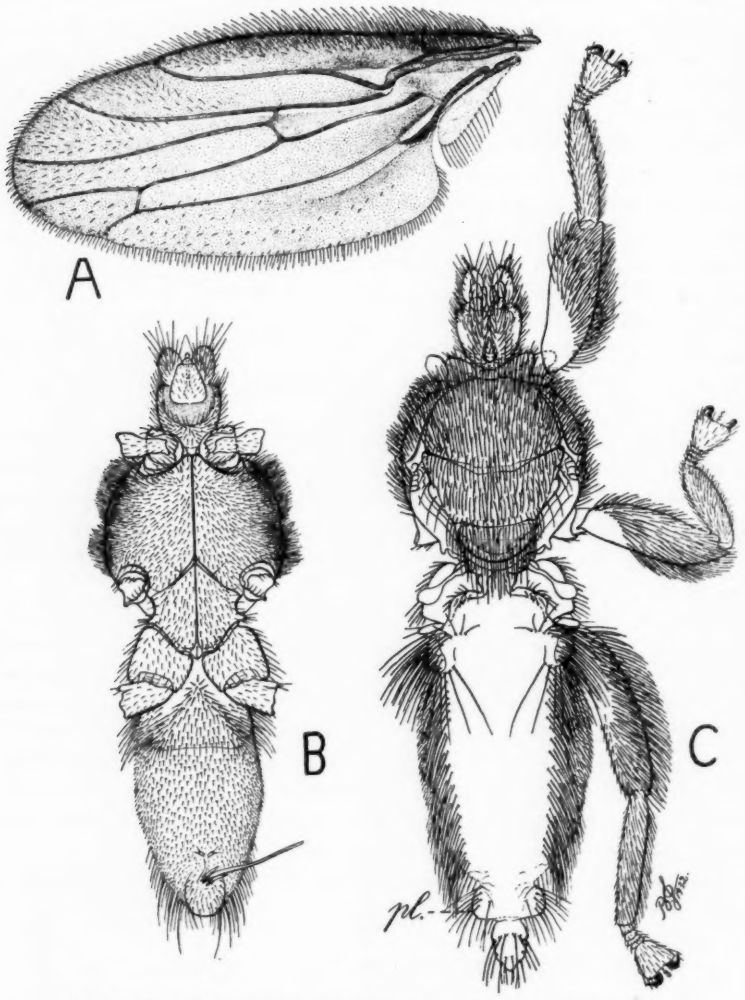

Fig. 6. *Nycteribosca amboinensis* Rondani. A, wing; B, ventral view of body of male; C, dorsal view of female.

distinct; postvertex piriform, with a few small setae, and many small setae behind the postvertex on the occiput; eyes small; postgenae with very many thin setae. Palps slightly longer than broad, with thin long setae. Theca conical, as long as broad at the base.

Thorax as long as or a little longer than broad; prescutum and scutum very densely covered with thin long setae, which in some specimens almost conceal the dorsal surface of the thorax; scutellum rounded, with very many upwardly directed thin, long setae; mesopleurae and pteropleurae with numerous but shorter setae than those of the scutum; each metapleura with a small knob-like process projecting posteriorly. The ventral surface of the thorax bears numerous very small setae. Legs stout, dorsal surface of femora with many very long, thin setae, the ventral side with many very small setae. The tibiae bear numerous very thin setae; the ventral surface of coxa and trochanter of the hind legs densely covered with very small setae.

Wings 2·5–2·7 mm. long. Second longitudinal vein very gently bent towards costal margin; third and fourth longitudinal veins divergent near the apex of the wing; distal end of the alula broadly rounded. Surface of the wing very setose; the setae especially very numerous in humeral costal and marginal cells; they are very long near the costal margin in the basal part and become gradually shorter towards the apex of the wing.

Abdomen with many very long setae arising on each side of the bare part of the dorsal surface; ventral surface with numerous very small setae. In the most posterior part of this surface the setae become more or less abruptly stronger and longer. Abdominal plates with very long setae, but these are not as numerous as those of *N. pretiosa*. Surface of the tergite-like part of the abdomen with many very long setae. Apical cone of the abdomen of the female much longer than broad, with four dorsal, two lateral and two ventral long setae and a few small ones.

Hypopygium of the male spherical when examined from below, with moderately long setae arising from the posterior part of its dorsal and lateral surfaces; its ventral surface covered with small setae.

Specimens of this species even from the same locality vary slightly in their structure and chaetotaxy. It has been recorded from Amboyna, Burma, Helwak, Nicobar Islands and Philippine Islands, from *Hipposideros larvatus* Horsfield, *Miniopterus schreibersi* Kuhl., and *M. eschscholtzii*.

Nycteribosca surcoufi Falcoz, 1923

(Fig. 7)

Two type specimens, ♂ and ♀, were examined from the collection of Dr Falcoz. This species very much resembles *N. amboinensis*, from which, however, it can be separated by its bombiform vertex, rounded theca of the labium and pointed distal end of the alula.

Body 3 mm. long (on slide). Head very convex, its vertex bombiform; eyes small; each laterovertex with long setae; mediovertex very narrow; postvertex piriform, with longish setae; a few small setae on the occiput behind the postvertex; postgenae with very many longish setae. Palps as long as broad. Theca of the labium rounded laterally; labella very small.

Thorax longer than broad when examined from above; prescutum and scutum very densely covered with long setae; scutellum rounded, with very many long, erect setae; mesopleurae and pteropleurae with numerous setae slightly shorter than those of the scutum; each metapleura with small, conical process. Ventral surface of the thorax beset with many very small setae. Legs stout; the dorsal surface of femora densely covered with very long setae, the ventral surface with very many small setae; tibiae densely covered with very small setae, which are slightly longer on the dorsal surface; the ventral side of coxae and trochanters of the hind legs densely beset with very small setae.

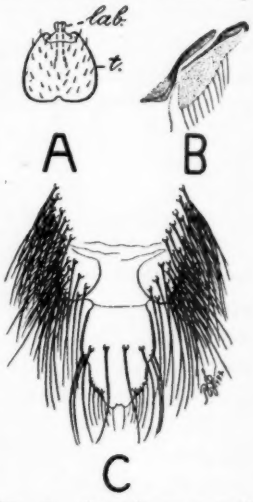

Fig. 7. *Nycteribosca surcoufi* Falcoz. A, ventral view of labella (*lab.*) and theca (*t.*); B, alula; C, posterior end of abdomen of female.

Wings 2·6 mm. long; their venation and chaetotaxy are the same as those of *N. amboinensis*. Alula with pointed distal end.

Abdomen of the female with very numerous long, strong setae on each side of the bare part of the dorsal surface; ventral surface very densely covered with very small setae; lateral plates of the posterior part of the abdomen very distinct, with many long, strong setae; posterior cone of the abdomen twice as long as broad, larger than that of *N. amboinensis* (Fig. 7 C).

Abdomen of the male with not so many long, strong setae on each side of the bare part of the dorsal surface. The hypopygium resembles that of *N. amboinensis*.

New Caledonia, from undetermined host.

Nycteribosca modesta sp.n.

(Fig. 8)

Three specimens were examined, the type, ♀, and two paratypes, ♀♀, from the collection of the Cambridge Museum. In its general appearance this species slightly resembles *N. rouxi*, from which, however, it can be easily separated by its smaller size and the absence of the process from the scutellum. From all the other species which have the third and the fourth longitudinal veins divergent at the apex of the wing, it can be separated by the structure of the fourth longitudinal vein. The basal part of this vein is distinctly longer than the part between the anterior and the posterior cross-veins.

Body more or less darkly pigmented, 2·4 mm. long (on slide). Head not darker than the body; each laterovertex with moderately long setae; mediovertex narrow; postvertex ovoid, with many setae; a few very small setae on the occiput behind the postvertex; postgenae not very setose. Palps as long as broad, rounded. Theca of the labium longer than broad.

The thorax distinctly broader than long; prescutum and scutum bear very many long setae, these are longer in the anterior part of the prescutum; scutellum crescent-shaped, with small setae along its anterior and posterior borders and many long setae arising from the middle; mesopleurae and pteropleurae with many setae of the same length as those of the scutum. The ventral surface of the thorax bears many very small setae; these are nearly as

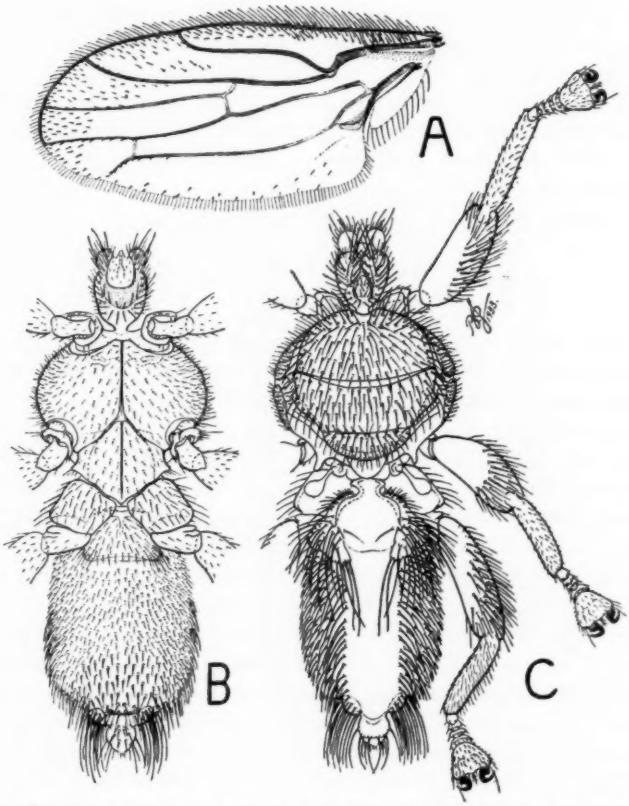

Fig. 8. *Nycteribosca modesta* sp.n. A, wing; B, ventral, and C, dorsal views of female.

long as the distance between them. Each metapleura with a long process. Legs stout; front coxae widely separated; the dorsal surface of femora with many long, strong setae, the ventral side with setae of the same size as those on the ventral surface of the thorax; tibiae beset with very small setae, the dorsal surface of the hind tibiae with much longer setae.

Wings 2 mm. long; third and fourth longitudinal veins divergent near the apex of the wing; the basal part of the fourth longitudinal vein distinctly longer than the part between the anterior and the posterior cross-veins. Distal

end of alula narrowly rounded. Many long setae on and near the costal margin; these setae are long near the basal part of the wing, but become gradually shorter towards the apex.

Abdomen with very many long, strong, dark setae on each side of the bare part of its dorsal surface; the spirally arranged barbs of these setae are very distinct. Towards the ventral surface the setae become much smaller; the ventral surface bears numerous small setae arising from the broad base. In the posterior part of the ventral surface, in front of the ventral abdominal plate, the setae become stronger and longer. Lateral abdominal plates distinctly sclerotised and bearing very many strong, long setae. Posterior cone of the abdomen small, its basal part spherical when viewed from above, with six long setae arising from its posterior margin; its ventral surface with two moderately long and a few small setae. Basal tergite and sternite-like parts of the abdomen very distinct on account of their pigmentation.

From *Rhinolophus rouxi* Temm., Alutnevara, Ceylon.

Nycteribosca rouxi Falcoz, 1923

(Fig. 9)

One type specimen, ♀, from the collection of Dr Falcoz was examined. This species is easily separated from all the others by the presence of a very darkly pigmented posteriorly pointed process of the scutellum.

Body darkly pigmented, 3·1 mm. long (on slide).

Head not darker than the thorax; each laterovertex with strong setae; mediovertex narrow; postvertex more or less ovoid, with a few setae; three small setae arise from the occiput, behind the postvertex; eyes small, distinct; postgenae with many small setae, the setae arising from the inner margin at each postgena being slightly longer. Palps longer than broad. Theca of the labium as long as broad, ovoid; labella very small.

Thorax very darkly pigmented, distinctly broader than long; prescutum and scutum bear many strong setae, the setae of the anterior part of the prescutum being distinctly longer; scutellum with a pointed process which is very darkly pigmented and bent ventrally; the surface of the scutellum has very long setae arising from the middle and many shorter setae near the borders. Each mesopleura and pteropleura with setae of the same length as those of the scutum; each metapleura with conical process. Ventral surface of the thorax with very small setae. Legs strong; the dorsal surface of femora with many strong, long setae, the ventral surface with very small setae; tibiae sparsely beset with small setae; the setae are longer on the dorsal surface, especially on the hind tibiae.

Wings 3 mm. long. Third and fourth longitudinal veins slightly divergent near the apex of the wing; second longitudinal vein gently bent towards the costal margin. Alula with broadly rounded distal end; the basal part of the costal margin with long setae, which become gradually shorter towards the apex of the wing; marginal, submarginal and posterior cells not very setose.

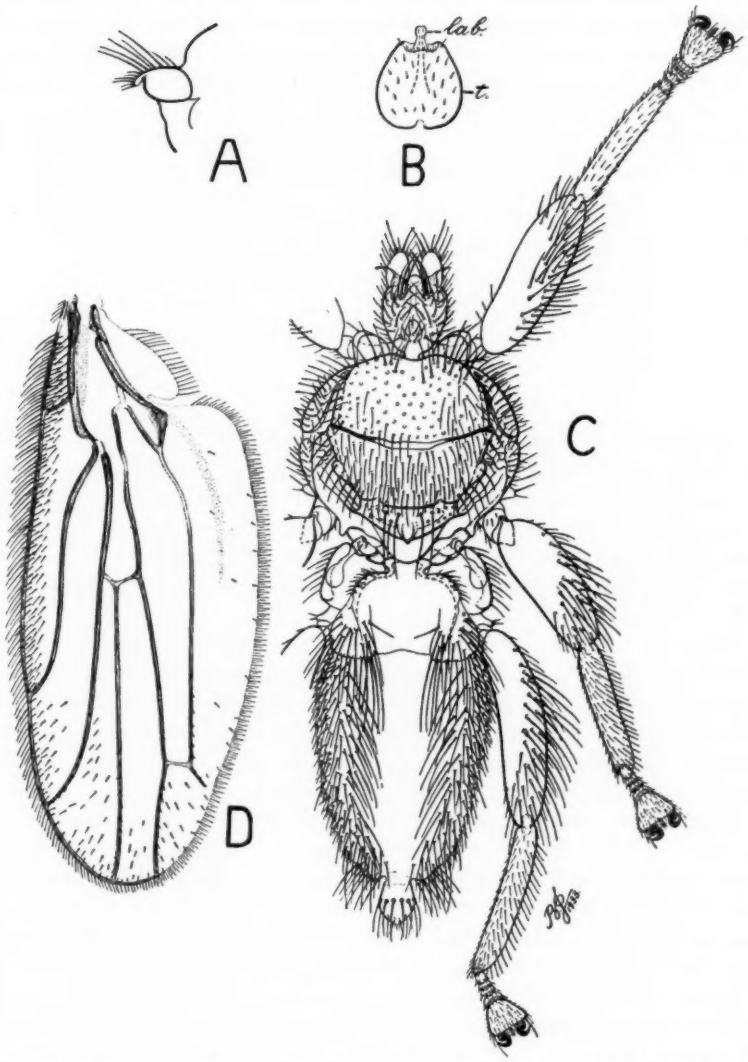

Fig. 9. *Nycteribosca rouxi* Falcoz. A, lateral view of scutellum; B, ventral view of labella (*lab.*) and theca (*t.*); C, dorsal view of female; D, wing.

Abdomen with very many strong setae on each side of the bare middle part of its dorsal surface; towards the ventral surface the setae become gradually smaller. Ventral surface with numerous very small setae; in the most posterior part of this surface the setae become more or less abruptly very stout and resemble spines, the setae immediately in front of the ventral abdominal plate as strong as those on this plate. Lateral plates of the abdomen very distinct with many long setae. Posterior cone of the abdomen longer than broad, pointed posteriorly, with four dorsal, two lateral and four ventral strong setae.

From *Notopteris neocaledonica* Trouess., from New Caledonia.

Nycteribosca africana (Walker, 1849) Speiser, 1900

Strebla africana Walker, 1849
Raymondia kollari Frauenfeld, 1855 (*nec* Schiner, 1868)
Strebla kollari Kolenati, 1856
Brachytarsina kollari Kolenati, 1863
Nycteribosca kollari Speiser, 1900

(Fig. 10)

Very many specimens of this fly were examined from different localities of the Mediterranean part of the Palaearctic region and the Ethiopian region. All the specimens were compared with Walker's type specimen of *N. africana*, and it became obvious that they were identical in every respect, and should be regarded as one species. Since Walker's paper was the earlier published, the name of *N. kollari* Frauenfeld, 1855 becomes a synonym of *N. africana* Walker, 1849.

Body 2·3–2·6 mm. long (on slide). Head not darker than the body; mediovertex narrow, not quite distinct; each laterovertex has, besides three long setae arising from its anterior part, many shorter setae; postvertex distinctly ovoid, with many small setae; a few small setae behind the postvertex, on the occiput; genae with many small setae; eyes small. Palps slightly longer than broad. Theca of the labium more or less conical.

Thorax broader than long; prescutum and scutum with many strong setae, the setae distinctly longer in the anterior part of the prescutum; the dorsal part of the mesopleurae and pteropleurae with slightly shorter setae; scutellum very broadly rounded, with a few very long and many much shorter setae; each metapleura with a knob-like process. Ventral surface of the thorax with many very small setae. Legs strong; the dorsal surface of femora with many long setae, the ventral with small setae; tibiae beset with small, thin setae; the ventral surface of coxae and trochanter of the hind legs densely covered with small setae.

Wings 2·6–2·9 mm. long. Second longitudinal vein very gently bent towards costal margin; third and fourth longitudinal veins very divergent near the apex of the wing. Alula with its distal end nearly pointed; costal margin of the wing fringed with long setae, these setae very long in the basal part, but becoming gradually shorter towards the apex of the wing.

Abdomen with many long, strong setae on each side of the bare part of its dorsal surface, lateral surfaces with much smaller setae which gradually become much smaller towards the ventral surface. Ventral surface with many very small setae, each arising from the large, rounded base. Lateral plates of the

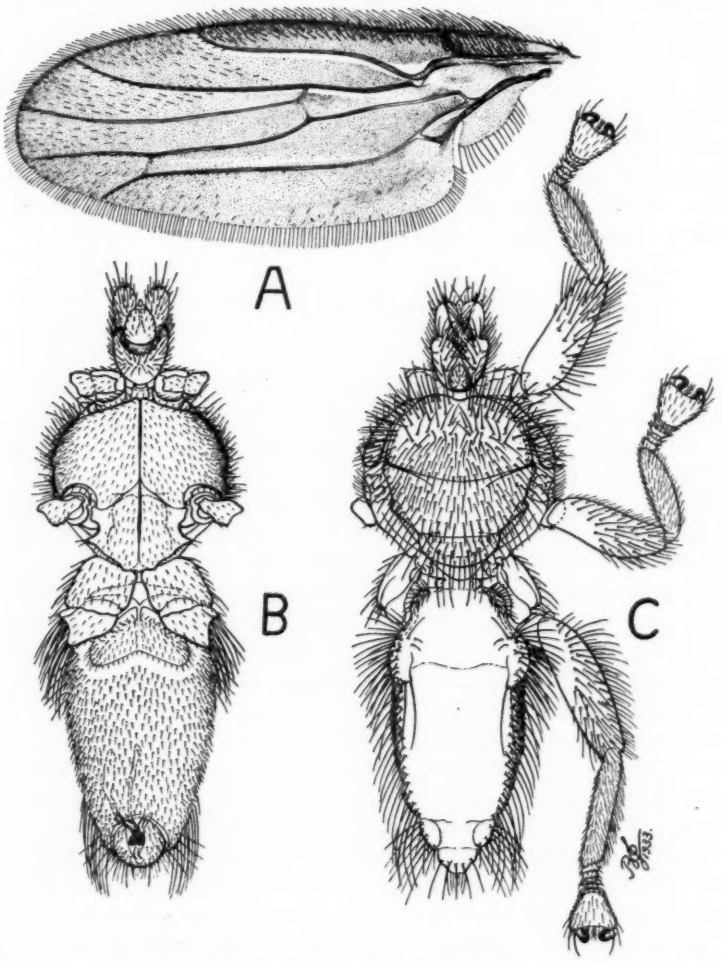

Fig. 10. *Nycteribosca africana* Walker. A, wing; B, ventral view of male; C, dorsal view of female.

posterior part of the abdomen distinct, with very long setae in their dorsal parts and small ones in their ventral parts. Posterior cone of the abdomen of the female broader than long, with three or four dorsal, two lateral and two ventral long and a few small setae. The basal parts of the small setae of the ventral surface of the abdomen of the male much smaller. Hypopygium semispherical when

examined from above, with a few longish setae arising from its lateral surfaces and the posterior part of the dorsal surface; its ventral surface bears small setae. Apodeme of the aedeagus longer than the other apodeme, spatulated.

The specimens of this species exhibit a slight variation in their chaetotaxy and the shape of the wings. This species has a very wide geographical distribution, it has been recorded from Portugal, South of France, Sardinia, Algeria, Tunis, Egypt, Cyprus, Palestine, British Somaliland, and as far south as Pretoria; it parasitises the following eleven species of bats: *Rhinolophus blasii* Peters, *R. ferrumequinum* Schreber, *R. meheleyi* Matschie, *R. hipposideros* Bechstein, *R. euryale* Blas., *R. geoffroyi* Smith, *R. capensis* Licht., *Asellia tridens* Geoffroy, *Miniopterus schreibersi* Kuhl., *Rhinopona microphillum* Geoffroy and *Vespertilio murinus* Linn.

Nycteribosca buxtoni Falcoz, 1927

(Fig. 11)

Two specimens were examined, the type, ♀, and the paratype, ♂, from the collection of the British Museum (Natural History). This species resembles *N. africana*, from which it can be easily separated by the characters shown in the key.

Body 2·5 mm. long (on slide). Head not darker than the body, very broad in its anterior part; eyes small, distinct; each laterovertex has, besides three long setae in its anterior part, longish setae; mediovertex distinct, more or less broad; postvertex longer than broad, its anterior end broadly rounded, the posterior end pointed; two small setae behind the postvertex, on the occiput; each postgena with many small setae. Palps longer than broad, rounded anteriorly. Theca of the labium as long as broad, with broadly rounded anterior end. Labella very small.

Thorax broader than long when viewed from above; prescutum and scutum with strong setae, the setae arising from the anterior part of the prescutum being the longer; mesopleurae and pteropleurae with slightly shorter setae than those of the prescutum; scutellum triangular, with setae of the same length as those of the scutum; metapleurae without process. Ventral surface of the thorax with many very small setae. Legs strong; the dorsal surface of femora with long setae, the ventral with small setae; tibiae beset with small very thin setae.

Wings 2 mm. long. Second longitudinal vein gently bent towards costal margin; third and fourth longitudinal veins strongly divergent near the apex of the wing. Distal end of the alula very narrowly rounded; costal margin fringed with long setae, these setae being very long in the basal part of the wing and becoming gradually shorter towards the apex.

Abdomen with many long, strong setae on each side of the bare part of its dorsal surface; the lateral surface with much shorter setae. Ventral surface bears numerous, very small setae which in the most posterior part of the

abdomen become longer. The posterior cone of the abdomen of the female is shown on the drawing (Fig. 11 C and E); it has four dorsal, two lateral and two ventral long setae, its ventral surface and the posterior margin having a few small setae.

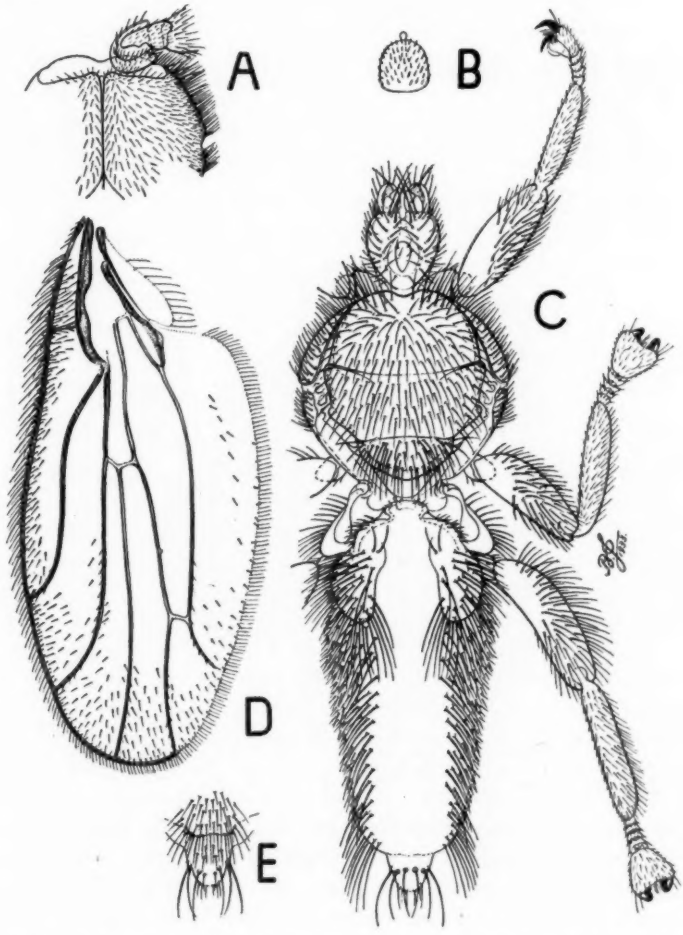

Fig. 11. *Nycteribosca buxtoni* Falcoz. A, sternopleura; B, ventral view of labella and theca; C, dorsal view of female; D, wing; E, posterior cone of abdomen of female.

The ventral surface of the abdomen of the male bears smaller setae. Hypopygium semispherical, with long setae arising from its lateral surfaces; its ventral surface bears much smaller setae. In the specimen examined the hypopygium was damaged and the apodeme of the aedeagus missing, I was

therefore unable to compare the length of the apodemes with regard to each other.

From *Emballonura semicaudata* Peale, Malololelei, Upolu, Samoa.

Nycteribosca speiseri sp.n.

(Fig. 12)

One type specimen, ♀, was examined, from the collection of the Cambridge Museum.

This new species resembles *N. pygialis*, from which it can be easily separated by its triangular scutellum, the curvature of the first longitudinal vein and the length of the anterior cross-vein with regard to the basal pale part of the third longitudinal vein.

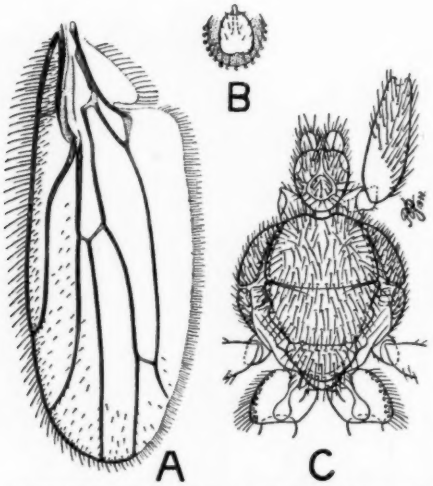

Fig. 12. *Nycteribosca speiseri* sp.n. A, wing; B, theca and labella; C, dorsal view of head and thorax.

Body 2·6 mm. long (on slide). Head not darker than the body, of the same shape as that of *N. pygialis*; eyes large, not very distinct; each laterovertex has, besides three long setae arising from its anterior part, smaller setae; mediovertex narrow, distinct; postvertex circular when viewed from above, with small setae, its posterior edge indistinct; occiput with a few small setae behind the postvertex; the surface of postgenae with many small setae; slightly longer setae arise from their inner margin. Palps as long as broad, rounded anteriorly. Theca of the labium barrel-shaped; labella very small.

Thorax nearly as long as broad; prescutum and scutum with many moderately strong setae; scutellum triangular, with setae of the same length as those of the scutum; the surface of mesopleurae and pteropleurae with slightly shorter setae than those of the scutum; metapleura without conical process.

Ventral surface of the thorax beset with very small setae. Legs strong; the dorsal surface of the femora with long setae, the ventral with very small setae; tibiae beset with very small setae; the ventral surface of coxae and trochanters of the hind legs with very small setae.

Wings 2·3 mm. long, rather narrow; first longitudinal vein sharply bent towards costal vein in its basal part, then going nearly parallel with that vein; second longitudinal vein gently bent towards costal margin; third and fourth longitudinal veins parallel near the apex of the wing; the basal pale part of the third longitudinal vein much shorter than the anterior cross-vein. Anterior margin of the wing with very long setae, especially in its basal part; the surface of wings not very setose. Distal end of the alula rounded.

Abdomen with many long setae on each side of the bare part of its dorsal surface; the ventral surface densely beset with very small setae. I could not study the most posterior part of the abdomen because it was very much distorted.

From *Hipposideros lankadiva* Kelaart, Peradenia, Ceylon.

Nycteribosca pygialis sp.n.

(Fig. 13)

One type specimen, female and nine paratypes, males and females were examined from the collection of the Cambridge Museum.

This is a very distinct species. From all the other species, in which the third and fourth longitudinal veins are parallel, except *N. speiseri* sp.n., it can be separated by its rounded, very distinct postvertex and the large eyes; from *N. speiseri*, by its very broadly rounded scutellum and the curvature of the first longitudinal vein.

Body 2·3 mm. long (on slide), rather darkly pigmented. Head not darker than the body; eyes large, distinct, not projecting laterally; each laterovertex has, besides three long setae arising from its anterior part, slightly shorter setae; mediovertex narrow, distinct; postvertex circular when viewed from above, very distinct, with small setae; occiput with a few small setae behind the postvertex; the surface of postgenae with small setae; the setae arising from the inner border slightly stronger. Palps nearly as long as broad, rounded anteriorly, with rather strong setae arising from their ventral surface. Theca of the labium longer than broad, labella very small.

Thorax spherical; the surface of prescutum and scutum with strong setae, and two long setae arise from the middle of the scutum; scutellum very broadly rounded, with its posterior margin nearly parallel to the anterior one, and with five or six very long and many very much smaller setae arising from its surface; the dorsal parts of mesopleurae and pteropleurae bear small setae, except near the margins, where the setae are as long as those of the scutum. Ventral surface of the thorax beset with many very small setae; each metapleura with rather large conical process. Legs not very strong; the dorsal surface of femora beset

with strong setae, the ventral with small setae; tibiae densely beset with very thin, small setae.

Wings 2·5 mm. long. First longitudinal veins gently bent towards costal margin; distal part of second longitudinal vein rather sharply bent towards

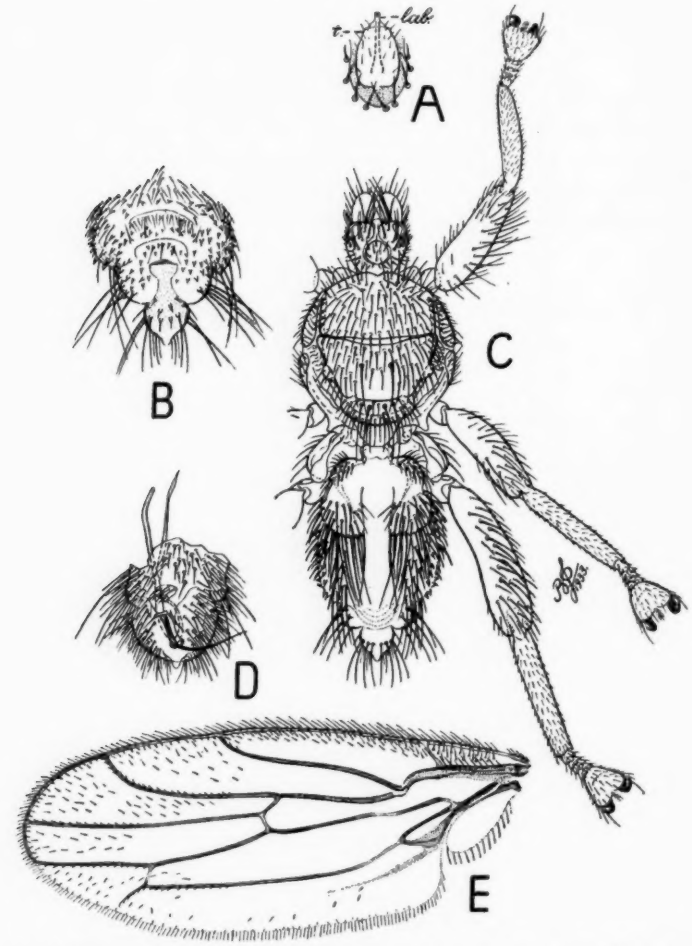

Fig. 13. *Nycteribosca pygialis* sp.n. A, ventral view of labella (*lab.*) and theca (*t.*); B, ventral view of posterior end of abdomen of female; D, same of male; C, female; E, wing.

costal margin; third and fourth longitudinal veins parallel near the apex of the wing; the basal pale part of the third longitudinal vein longer than the anterior cross-vein; anterior margin of the wing fringed with rather short setae. Surface of the wing not very setose. Distal end of the alula very broadly rounded.

Abdomen of the female with very long setae arising on each side of the bare part of its dorsal surface; the lateral surfaces with much shorter setae and small, very broad-based spine-like setae in the posterior part. Ventral surface of the abdomen with small setae in its anterior part and with spine-like setae in the posterior part. Lateral plates of the posterior part of the abdomen distinct, with very strong, long setae. Posterior cone of the abdomen dome-shaped, with six dorsal, two lateral and two ventral long setae. There are a few small, broad-based setae on its ventral surface.

The male abdomen differs from that of the female in the following respects: Its ventral surface is beset with small setae, there are no spine-like setae. Hypopygium very large, semispherical, with thin, long setae arising from its dorsal and lateral surfaces; its ventral surface bears much smaller setae. Apodeme of the aedeagus longer than the other apodeme.

From *Hipposideros lankadiva* Kelaart, Gonagama Estate, Kitulgala, Ceylon, 600–700 ft., collected by Captain W. W. A. Phillips.

Nycteribosca alluaudi Falcoz, 1923

(Fig. 14)

Two specimens, ♂ and ♀, were examined from the collection of the British Museum (Natural History).

Body 3 mm. long (on slide). Head not darker than body; eyes small, distinct; each laterovertex having, besides three long setae arising from its anterior part, many slightly shorter setae; mediovertex very narrow; postvertex more or less triangular, with very small setae; two very small setae on the occiput, behind the postvertex; genae with moderately strong setae along their inner border and many small setae arising from the surface. Palps as long as broad, rounded anteriorly. Theca of the labium large, conical, longer than broad; labella very small.

Thorax broader than long; prescutum and scutum with many strong setae; the surface of mesopleurae and pteropleurae with shorter setae; scutellum more or less triangular, with setae of the same length as those of the scutum; each metapleura with conical process. The ventral surface of the thorax bears very many very small setae. Legs strong; the dorsal surface of femora with strong setae, the ventral with many very small setae; tibiae beset with very small setae; the ventral surface of coxae and trochanters of the hind legs with very small setae.

Wings 3 mm. long. The distal part of second longitudinal vein sharply bent towards costal margin; third and fourth longitudinal veins parallel near the apex of the wing; the basal pale part of the third longitudinal vein much shorter than the anterior cross-vein; anterior margin of the wing fringed with short setae. Surface of the wing moderately setose. Distal end of alula very broadly rounded.

Abdomen with many strong, long setae on each side of the bare part of its

dorsal surface; the ventral surface with numerous very small setae. Posterior cone of the abdomen of the female with four dorsal, two lateral and four ventral long setae; there are a few small setae on its ventral surface.

From *Taphozous hildegardeae* Thomas, from British East Africa and Alexandria, Egypt.

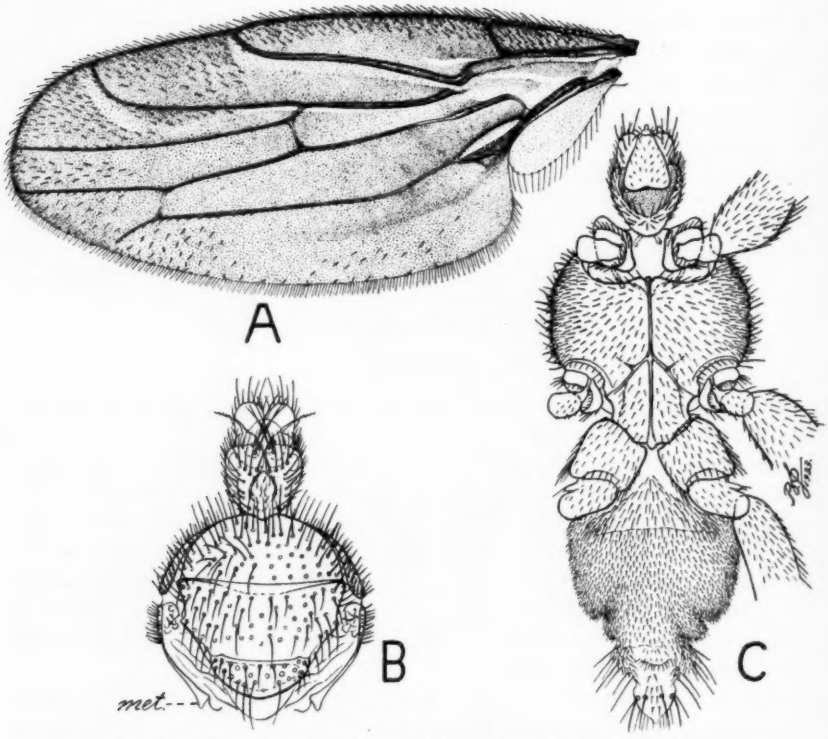

Fig. 14. *Nycteribosca alluaudi* Falcoz. A, wing; B, dorsal view of head and thorax; C, ventral view of body of female.

Nycteribosca caudata sp.n.

(Fig. 15)

One type specimen, ♀, examined, from the collection of the British Museum (Natural History). This species very much resembles *N. alluaudi* in its general appearance, but it can be easily separated from that species by the following distinctive characters: the ovoid theca of the labium; the very broadly rounded scutellum; the posterior cone of the abdomen of the female, which has many more long setae; and, finally, by the basal pale part of the third longitudinal vein, which is as long as the anterior cross-vein.

Body 2·2 mm. long (on slide). Head not darker than the body; eyes small,

distinct; each laterovertex with moderately strong setae practically of the same length; mediovertex narrow, quite distinct; postvertex more or less triangular, with a few small setae; a few very small setae on the occiput, behind the postvertex; genae with small setae; stronger setae arise from their inner border. Palps slightly longer than broad. Theca of the labium ovoid.

Thorax spherical when viewed from above, nearly as long as broad; the surface of prescutum and scutum bears many strong setae; two much stronger

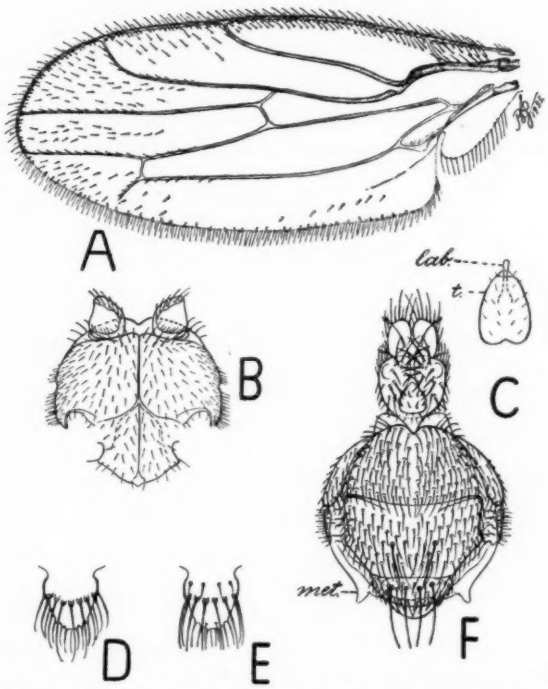

Fig. 15. *Nycteribosca caudata* sp.n. A, wing; B, ventral view of thorax; C, ventral view of labella (*lab.*) and theca (*t.*); D, dorsal, and E, ventral views of posterior cone of abdomen of female; F, dorsal view of head and thorax.

setae arise from the middle of the scutum; the surface of mesopleurae and pteropleurae with shorter setae than those of the prescutum; scutellum very broadly rounded, crescent-shaped, with many small and four very long setae. Ventral surface of the thorax with small setae. Legs strong; the dorsal surface of femora with strong setae, the ventral surface with very small setae; tibiae beset with many very small setae.

Wings 2·5 mm. long. Second longitudinal vein sharply bent towards costal margin in its distal part; third and fourth longitudinal veins parallel near the apex of the wing; the basal pale part of third longitudinal vein as long as the

anterior cross-vein; anterior margin of the wings fringed with short setae; the surface of wings moderately setose. The distal end of alula broadly rounded.

Abdomen with many long setae on each side of the bare part of its dorsal surface; the ventral surface beset with very small setae. Posterior cone of the abdomen of the female ringed round the middle with many long setae; there are four very small setae in the basal part of its ventral surface and a few very thin setae on the posterior margin.

From *Taphozous melanopogon* Temm., Mandalay, Burma.

Nycteribosca diversa Frauenfeld, 1856

(Fig. 16)

This doubtful species is, according to its author, smaller than *R. africana* Walk., but has the same arrangement of wing venation except that the second

Fig. 16. Wing of *Nycteribosca diversa* Frauenfeld. (After Frauenfeld.)

longitudinal vein has a branch enclosing the triangular space which has its base on the anterior margin of the wing. In other respects it resembles *R. africana*.

From *Cynonyctaris aegyptiaca*, Egypt.

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A NEW SPECIES OF THE GENUS *NYCTERIBOSCA* (DIPTERA PUPIPARA, STREBLIDAE)

By B. JOBLING

From the Wellcome Bureau of Scientific Research, London

(With 1 Figure in the Text)

Nycteribosca minuta sp.n.

Three specimens were examined, the type, female, and two paratypes, male, collected by Mr B. A. Lever, from a small bat in the Russell Islands, Solomon Islands. They were sent to me by Sir Guy A. K. Marshall, whom I wish to thank for allowing me to describe them.

This new species differs from all other species of the genus in having extremely small, almost indistinguishable eyes. In its general appearance it very much resembles *N. modesta* Jobling (1934), but differs from it by its much smaller size, distinctly ovoid postvertex which is separated from the occiput, and by the presence of a row of long, curved, spine-like setae in the posterior part of the ventral surface of the abdomen of the female (Fig. 1 D). The wing venation of the new species is also different from that of *N. modesta*. The first longitudinal vein is very slightly curved in the basal part, while the distal part of the second longitudinal vein is sharply bent towards the costal vein, and the basal part of the fourth longitudinal vein is about as long as the part of this vein which extends between the anterior and the posterior cross-vein.

Body 1·7–2 mm. long (on slide). Head not darker than the body and not very convex dorsally; eyes very small, almost inconspicuous; each lateravertex has three long setae arising from the anterior part, besides the slightly shorter setae; mediovertex very distinct; postvertex distinctly ovoid, separated from the occiput, with a few longish setae; no setae behind the postvertex on the occiput; postgenae with many small setae; their inner border fringed with slightly longer setae; palps slightly longer than broad. Theca of the labium somewhat conical, longer than broad; labella very small (Fig. 1 A).

Thorax broader than long. The prescutum and scutum bear many setae, which are much longer in the anterior part of the prescutum and the posterior part of the scutum; scutellum very broadly rounded, with very long setae arising from the middle and small setae in the anterior and posterior parts; mesopleurae and pteropleurae bearing setae of the same length as those of the posterior part of prescutum. The ventral surface of the thorax bears many very small setae. Legs strong.

Wings 1·5–1·7 mm. long. First longitudinal vein slightly curved in its basal part, distal part of second longitudinal vein sharply bent towards costal margin; third and fourth longitudinal veins slightly divergent near the apex

of the wing; basal part of the fourth longitudinal vein nearly as long as the part of this vein which is between the anterior and the posterior cross vein; surface of wings not very setose; costal margin fringed with long setae. Distal end of alula broadly rounded.

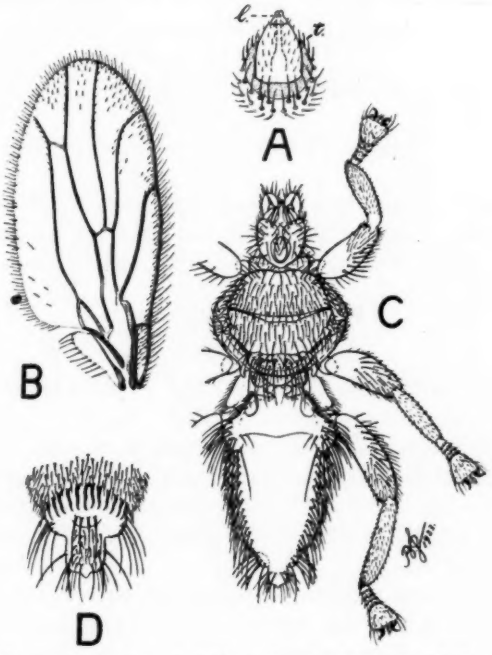

Fig. 1. *Nycteribosca minuta* sp.n. A, labium, showing labella *l.* and theca *t.*; B, wing; C, dorsal view of female; D, ventral view of posterior end of abdomen of female.

Abdomen with very many long setae on each side of the bare part of its dorsal surface; the ventral surface beset with small setae; in the posterior part of the ventral surface there is a row of very strong, curved spine-like setae. Lateral plates of the posterior part of the abdomen of the female with a few, and ventral plate with four long setae. Posterior cone of the abdomen of the female small, more or less cylindrical, with four dorsal, two lateral and two ventral long setae; there are also two moderately long and many very small setae on its ventral surface (Fig. 1 D).

Male differs from female as follows: there are no strong, curved spine-like setae on the posterior part of the ventral surface of the abdomen which ends in a large semispherical hypopygium.

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(*MS. received for publication* 8. XII. 1933.—Ed.)

THREE NEW SPECIES OF FLEAS FROM DUTCH NEW GUINEA

By MIRIAM ROTHSCHILD

(With 11 Figures in the Text)

In 1931 a number of fleas was received from Mr Georg Stein, obtained in Dutch New Guinea. Among them were the three species described below. We are greatly indebted to Mr Stein for having made this collection.

Stivalius anaxilas n.sp.

(Figs. 1, 2, 3)

A single male was obtained off *Mus* sp. in the Weyland Mountains (1500 m.). This species is most nearly related to *St. mordax* and *St. rectus*.

Head (♂, Fig. 1). Frons. More convex than in *St. rectus* and *St. mordax*. Anterior row of bristles situated nearer edge than in other species of the genus. Contains eight spiniform bristles. Second row contains six slenderer bristles. Between this row and antennal groove, about fourteen irregularly arranged long stout bristles.

Occiput. Second and third row of bristles set slightly more obliquely than in *St. rectus* and *St. mordax*. Small bristles round antennal groove less numerous.

Thorax. Pronotal comb with eighteen spines, longer than entire pronotum. Mesonotum with about thirty-six main bristles (both sides together). Mesoplura and mesosternum together, with twenty-four bristles (both sides together). The longest bristles in the posterior row on the meso- and metanotum are the same length as the spines of the pronotal comb. In *St. rectus* and *St. mordax* they are longer. Metepimeron with twenty-six main bristles (both sides together).

Abdomen. Tergites I–VII with two rows of bristles only. The description of the genus (Jordan and Rothschild, 1922) should be modified accordingly.

Legs (Fig. 2). First segment of fore-tarsus with one long stout bristle on posterior margin instead of three, as in *St. rectus* (Fig. 4) and *St. mordax*.

Modified segments (Fig. 3). Apex of VIIIth sternite triangular with tip rounded. About twenty-three large bristles and several smaller ones. In this specimen serrations of upper margin deeper than in *St. rectus* and *St. mordax*. Posterior margin of clasper with deep narrow sinus below short process (*P*). The two bristles at ventral apical angle of clasper set closely together. Ventral margin without incurving immediately in front of angle. Exopodite finger (*F*)

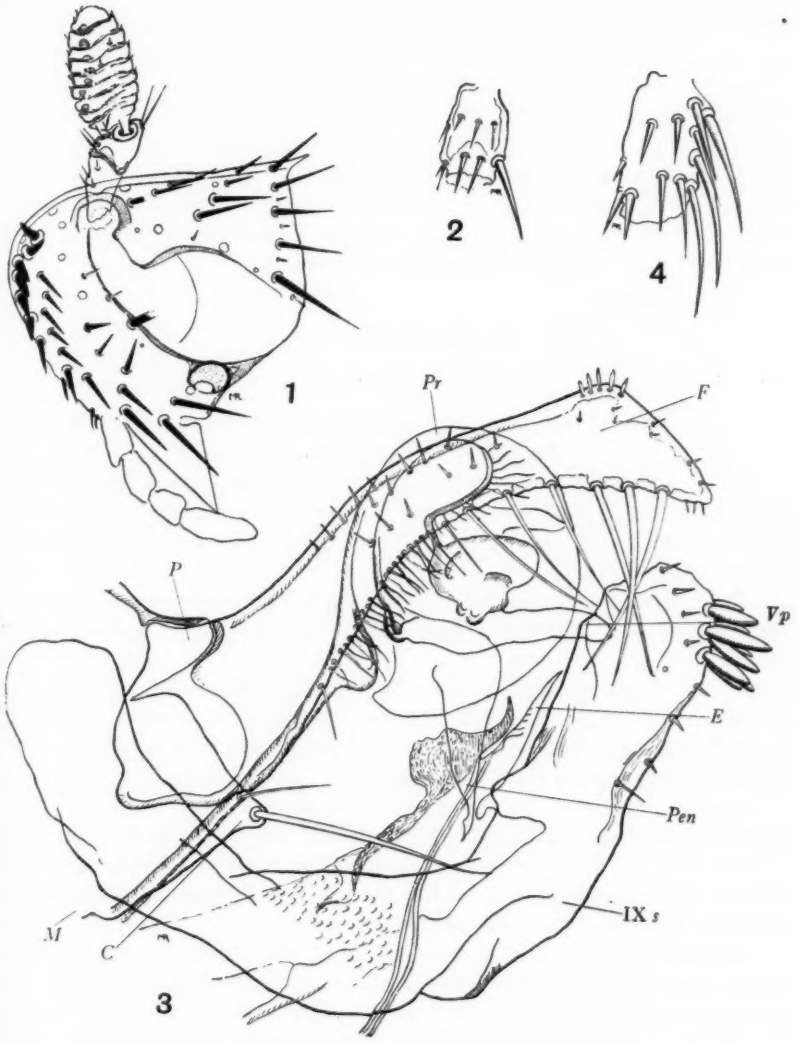

Fig. 1. Head of *St. anaxilas.*
Fig. 2. First joint of fore-tarsus of *St. anaxilas.* ♂
Fig. 3. Modified segments of *St. anaxilas.*
Fig. 4. First joint of fore-tarsus of *St. rectus.* ♂

Lettering: *A*, anus; *B*, bursa copulatrix; *C*, clasper; *E*, ejaculatory duct; *F*, exopodite finger; *M*, manubrium of clasper; *P*, process of clasper; *Pen*, penis; *Pyg*, pygidium; *Pr*, paramere of penis; *R*, receptaculum seminis; *St*, stigma cavity; *Sty*, stylet; *Vp*, ventral process of paramere.

with upper margin less convex than in *St. rectus* and *St. mordax* with apical portion narrower. Apex of IXth sternite more rounded. Not widened before apex as in *St. rectus* and *St. mordax*. Protrusion on upper margin near base of vertical arm more pronounced. Some of apical bristles spiniform. Ejaculatory duct with apex faintly ribbed. Very characteristic shape to sclerite anterior to end of duct. Bulb-shaped, prolonged posteriorly into narrow pointed process. Dorsal area of paramere narrower, not hood-like as in *St. rectus* and *St. mordax*. Apical margin evenly rounded. Membranous portion forming margin larger in comparison with upper portion. Process of paramere straight. Total length = 3·7 mm. Length of hind-tibia = 0·28 mm.

Stivalius spooneri[1] n.sp.

(Figs. 5, 6, 7, 8)

Three males and two females of this species, closely related to *St. lasiurus*, were obtained off *Phascologale* sp. in the Weyland Mountains (2000 m.).

Head. Frons. Shaped very like that of *St. lasiurus* but arrangement of bristles resembling that of *St. mordax* with anterior row nearer to margin of frons. About twenty to twenty-two stout bristles of varying size between margin and antennal groove.

Mouthparts. Maxillary palpus longer than in *St. lasiurus* reaching to four-fifths of fore-coxa. Small patches of sensory spines on fifth segment greatly reduced.

Thorax. Eighteen pronotal spines, longer than entire pronotum. Mesoplura and mesosternum together, with between twenty and twenty-four bristles (both sides together). Metepisternum six bristles (both sides together). Metepimeron thirty bristles (both sides together).

Abdomen. Apex of VIIth sternite does not project above antipygidial bristles. Lobe below antipygidial bristles nearly suppressed as in *St. lasiurus*.

Legs (Fig. 7). Fifth segment of fore-tarsus with first pair of lateral bristles shifted only slightly inwards. Third pair of lateral bristles shifted further inwards. In *St. lasiurus* the reverse is the case.

Modified segments (♂, Figs. 5 and 6). The pair of bristles at the ventral apical angle of clasper situated very close together, the smaller of the two almost as long as the other. Exopodite finger (*F*) much shorter than in *St. lasiurus*, but wider proximally. The whole inner surface except for a small dorso-apical area, and the base, densely covered with hairs. Seven pale spiniform bristles on apical dome, and row of long thin closely set bristles along dorsal margin, beginning some distance from dome. The base curved sharply upwards, strongly angulated with the rest of the exopodite.

IXth sternite very characteristic. Horizontal arm long. Vertical arm short with apex pointed and curved upwards, at some distance from apex, dorsal

[1] This species is named in honour of Mr G. M. Spooner.

margin enlarged into a triangular tooth. Five stout spiniform bristles arranged in a row along apical ventral margin. Dorsal margin of inner side of the sclerite bears three other teeth, more cylindrical in shape, and not pointed.

Xth sternite with terminal bristles reaching beyond end of exopodite on account of the shortness of the latter. In *St. lasiurus* they reach about three-

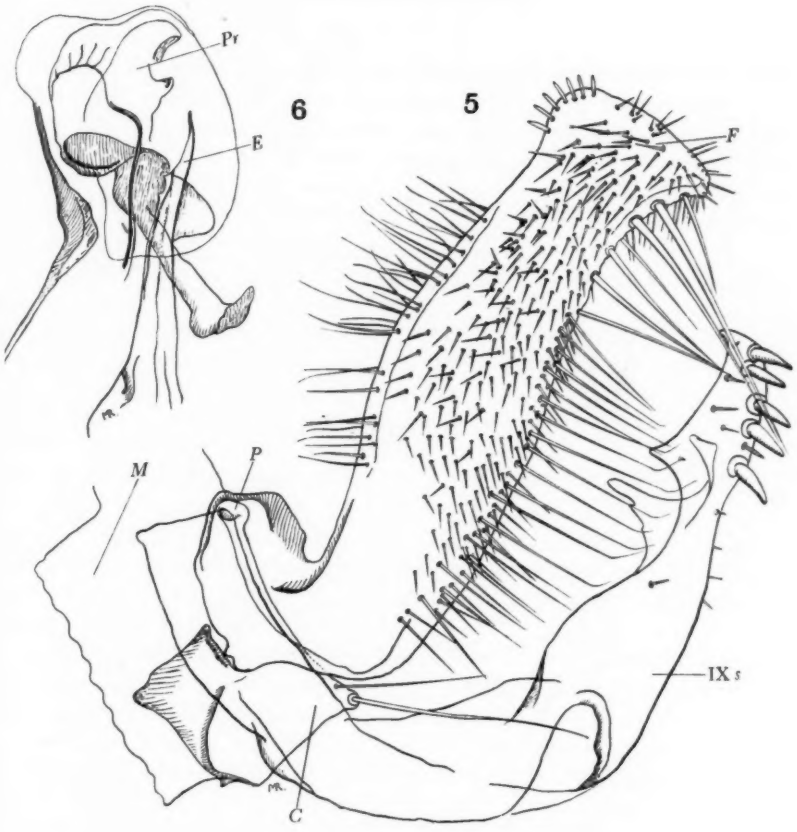

Fig. 5. Modified segments of *St. spooneri*.
Fig. 6. Penis of *St. spooneri*.

Lettering as in Figs. 1–4.

quarters of the length, and in *St. mordax*, *St. rectus* and *St. anaxilas* barely to the end. Ejaculatory duct very narrow at apex, with upper margin slightly denticulate but without processes. Paramere not rounded as in *St. lasiurus* but dorsally produced into a knob. Claw-like hook at posterior extremity.

♀, Fig. 8. VIIth sternite with upper margin concave instead of convex. No deep apical sinus as in *St. rectus* or *St. mordax* but only slight incurving. Lobe below this emargination rounded and projecting more than lobe above.

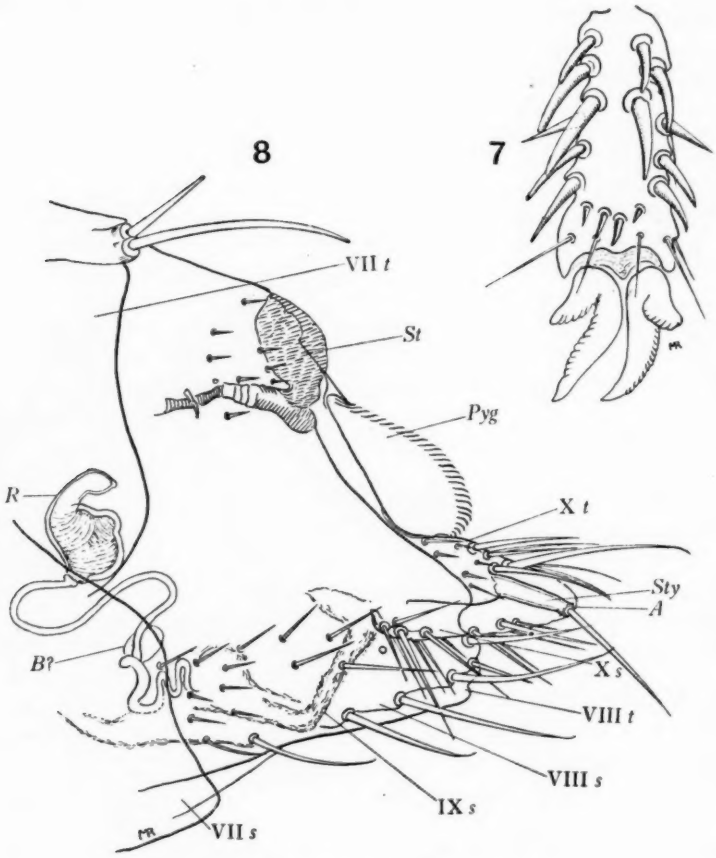

Fig. 7. Fifth joint of fore-tarsus of *St. spooneri*. ♂
Fig. 8. Modified segments of *St. spooneri*.

Lettering as in Figs. 1–4.

Bristles less numerous and slenderer than in *St. lasiurus*. Posterior row contains six stout ones. VIIIth tergite with ventral apical angle rounded off. Marginal bristle above this angle situated on short projection. Sinus above this bristle not so deep as in *St. lasiurus*. Stylet with the small ventral sub-apical bristle situated further from apex than the small dorsal bristle. Receptaculum seminis with body longer than broad. Hump on dorsal surface nearer to tail than orifice.

Orifice on a round projection. Tail shorter than body, twice as long as broad, with small terminal appendage. Duct long.

The condition of the bursa copulatrix in these specimens does not permit of a description.

Total length = (♂, ♀) 2·4 mm. Length of hind-tibia = (♂, ♀) 0·36 mm.

Acanthopsylla praxilla n.sp.

(Figs. 9 and 10)

A single female was taken off *Phascologale* sp. in the Weyland Mountains (2400 m.). It is closely related to *Acanthopsylla enderleini* Wagner 1933.

Head. Frons (Fig. 9) less rounded than in *A. enderleini* (Fig. 11) with about fifteen large to medium sized bristles in all. Anterior row of bristles situated farther from edge, containing one spine. (The description of the genus, by Jordan and Rothschild (1922), must be modified accordingly.) Three instead of two long fine bristles above it and one below.

Arrangement of occipital bristles as in *A. enderleini*. Eleven bristles round antennal groove. (Wagner mentions ten for his type specimen of *A. enderleini*, but specimens in the British Museum Collection possess twelve.)

Mouthparts. Maxillary palps do not reach the middle of the fore-coxa. In *A. enderleini* they reach well below the middle.

Thorax. Pronotal comb with eighteen spines, the longest of which is slightly shorter than the entire pronotum. Mesonotum as in *A. enderleini*, with three rows of bristles, anterior row somewhat irregularly arranged. Mesopleura and mesosternum (both sides together) with twelve stout bristles and six small ones. Metanotum with three small bristles each side, compared with two in *A. enderleini*. Metepisternum and metapleuron with one medium and one small bristle. Metepimeron with sixteen stout bristles and six small ones (both sides together).

Legs. The legs only differ from *A. enderleini* in the proportion of some of the joints. First joint of hind-tarsus slightly shorter instead of longer than hind-tibia. Relative length of hind-tarsal joints are 10 : 7 : 4 : 2 : 3.

Abdomen. Tergites I–VI with three rows of bristles (arranged as in *A. enderleini*) and one small bristle in front of these. VIIth tergite with four rows of bristles before antepygidial bristles. Counting both sides together, first row contains eight large, second row eight medium, third row six medium, fourth row four small, bristles.

Two antepygidial bristles on each side, the lower one barely twice as long as the upper. Ratio = 11 : 21. *A. enderleini* ratio = 2 : 5.

Sternites II–VI each with five stout bristles; three additional rows of five to three medium-sized bristles.

Modified segments (Fig. 10). VIIth sternite with eight stout bristles, in somewhat irregularly arranged row and twenty smaller bristles behind them. Marginal sinus deeper, triangular, with lobe below emargination protruding farther than lobe above. In *A. enderleini* the shape of the sinus is rather

variable. VIIth tergite and stigma cavity closely resembles *A. enderleini*. Body of receptaculum seminis only slightly longer than tail (ratio = 9 : 7), about one and a half times longer than broad. The bursa copulatrix in this specimen is too poorly preserved to describe. Total length of the specimen = 3·8 mm. Length of hind-tibia = 0·5 mm.

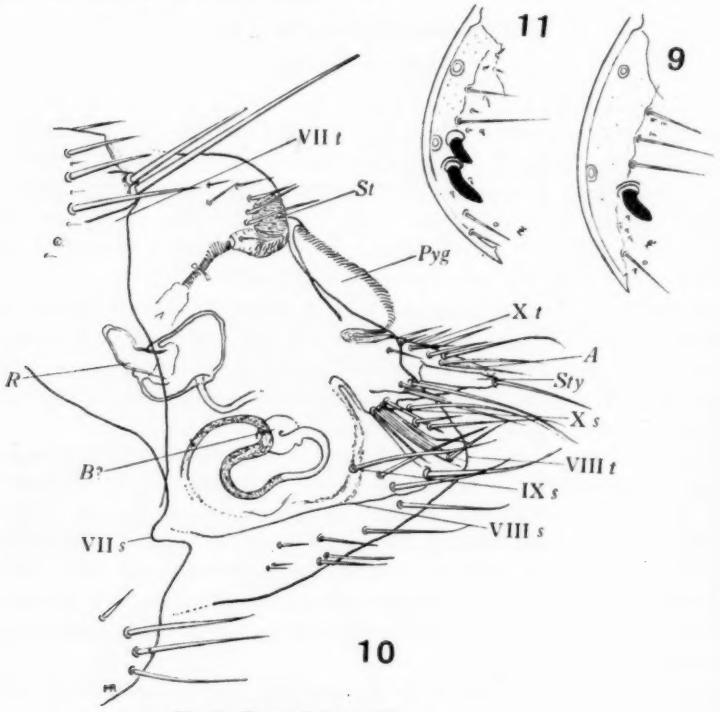

Fig. 9. Frons of *A. praxilla*.
Fig. 10. Modified segments of *A. praxilla*.
Fig. 11. Frons of *A. enderleini*.

Lettering as in Figs. 1–4.

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METAZOAN PARASITES FROM MICE IN THE ISLE OF LEWIS, OUTER HEBRIDES

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I. Introduction

Two kinds of mice occur in Lewis: the Hebridean Field Mouse (*Apodemus hebridensis* De Winton) and the House Mouse (*Mus musculus* L.), the latter living in the fields in summer. Brown Rats (*Epimys norvegicus* Berkenhout) also occur, both in villages and in Stornoway, and also on farms. The Pigmy Shrew (*Sorex minutus* L.) is also found, probably living both on the moors and in fields. There are no Common Shrews (*S. araneus* L.), Voles (*Evotomys* or *Microtus*) or Moles (*Talpa*). The results of a parasite survey of 10 *Apodemus*, 35 *Mus*, and 2 *Rattus*, and 11 nests of *Mus* are recorded below. The general object was to obtain information for comparison with similar surveys near Oxford (Elton, Ford, Baker and Gardner, 1931) and in Norwegian Lapland (Elton, 1934). The special idea underlying the Lewis survey was that a knowledge of the parasite fauna of *Apodemus* on the different islands of the British archipelago (especially the outer ring of Shetlands, Hebrides, Isle of Man, and Ireland), also on Iceland, would throw light upon the Pleistocene and recent faunal history of these islands. This enquiry is being carried out in co-operation with Mr D. Baden-Powell, who is studying the geological aspects of the problem, and whom I wish to thank for help in many ways during our expedition to Lewis in the summer of 1933. I have also to thank Mr Kenneth MacLean, a crofter at Brue, Barvas, for hospitality and much help, especially in sending me nests of House Mice. A second aim of the survey is to build up gradually a complete ecological survey of British rodent parasites in connection with the study of periodic epidemic disease among Voles, Mice and Shrews. *Apodemus* were extremely scarce, House Mice less so. It was realised that a study of House Mouse parasites was important in order to check up the introduction of parasites to the island by them. The identification of various groups has been undertaken by several experts, to whom I am indebted: Dr H. A. Baylis (parasitic worms); Mr J. M. Brown (Collembola and Psocoptera); Dr M. Cameron (beetles); and Miss Margaret Hora (mites), who is publishing a separate paper on the mites, the study of which is beset with many difficulties owing to the scattered literature and the scarcity of experts in this country. The full parasite data for each rodent or nest were recorded on a card, together with habitat, weight, sex, and also the number and weight of

embryos (if any). These records and my journal of field work are deposited in the Bureau of Animal Population. The fleas, mites and parasitic worms are being deposited in the British Museum (Natural History); the beetles and Collembola in the Hope Collection at Oxford.

II. Mice and rats

Fleas (*Siphonaptera*). The flea-rate was very low, only 16 being taken from a total of 10 *Apodemus* (4 taken alive), and 35 House Mice (6 taken alive). For neither species was the average more than one flea to two mice. The fact that they were mostly dead when examined is of course of significance; but comparisons of flea-rates on dead and living mice have shown this factor to be less important than is commonly supposed (Elton and others, 1931). The fleas, all taken near Stornoway, belonged to three species: *Ctenophthalmus agyrtes* Heller var. *celticus* Rothschild 1922 (2 on *Apodemus* and 11 on House Mice), *Typhloceras poppei* Wagner (1 on *Apodemus*), and *Ceratophyllus fasciatus* Bosc (2 on House Mice, and 4 on two Brown Rats, the latter from a Stornoway warehouse). All the mice were trapped in fields, usually some distance from buildings.

Lice (*Anoplura*). The complete absence of lice from all the mice may be significant, but larger collections are required to confirm this point.

Mites and ticks (*Acarina*). These formed the most numerous and in some ways the most important element in the parasitic fauna. Miss Hora's separate report should be consulted. Briefly, 7 species (including one skin-mite, *Notoedres* sp.) occurred on *Apodemus*, and 8 species of mite and 1 tick on *Mus*.

Parasitic worms (*Nematoda*). Three *Apodemus* were examined for liver and intestinal worms. Two from Stoneyfield Farm, near Stornoway, had female roundworms, *Syphacia ? stroma* (v. Linst., 1884)[1] in the small intestine. A third field mouse from the Castle Grounds in Stornoway had a male specimen of the small red coiled Nematode, *Nematospiroides dubius* Baylis, in the duodenum. Four *Mus* from Stoneyfield Farm were examined, but no liver or intestinal worms found.

III. House Mouse nests

Through the kind help of Mr Kenneth MacLean it has been possible to obtain 11 nests of House Mice from corn fields on some crofts at Brue, Barvas. The House Mice live largely out of doors in the fields in the summer, and most of the nests were obtained at the corn harvest. Identification was assured by noting the presence of young or old "grey mice with grey bellies" in the nests, or running from them. All the nests were made of grass, one or two also containing bits of paper. The nest fauna was collected by means of a Berlese funnel. Most of the nests were sent by post and examined three days after

[1] Recent work by Morgan (1932) suggests that there are two species of *Syphacia*, distinguished by a male character. No males were collected in Lewis so that the exact species is still doubtful. *S. stroma* appears to be confined to *Apodemus*.

capture. The results are given in Table I, with figures wherever complete counts of the animals could be made. One nest contained no fauna, and is omitted. Mites are separately recorded by Miss Hora (1934).

Table I

Nest No. (refers to card index no. in B.A.P.)	6	50	51	52	54	55	56	57	58	59
Date (August)	8th	26th	25th	25th	24th	24th	30th	30th	30th	30th
Fleas (Siphonaptera):										
Ctenophthalmus agyrtes Heller var. *celticus* Rothschild 1922	1	64	9	.	8	14	1	1	*	18
Typhloceras poppei Wagn.	.	.	.	.	.	2	.	.	.	.
Flea larvae	×	×	×	×	.	×	.	.	.	×
Springtails (Collembola):										
Folsomia fimetaria (L.)	.	.	×	.	.	.	×	.	.	.
F. quadrioculata (Tullb.)	.	.	.	.	.	.	×	.	.	.
Lepidocyrtus albus (Pack.)	.	.	×	.	.	.	.	.	.	.
L. cyaneus Tullb.	×	.	.	.	.	.	.	.	.	.
Pseudachorutes asigillatus Born.	.	.	.	.	.	.	×	.	.	.
Hypogastrura viatica (Tullb.)	.	.	.	.	.	.	×	.	.	.
Tomocerus minor (Lubb.)	×	.	.	.	.	.	.	.	.	.
Psocoptera:										
Liposcelis divinitoria (Mull.)	.	×	.	.	.	.	.	.	.	.
Beetles (Coleoptera):										
Amischa cavifrous Shp.	.	.	3	5	.	.	.	.	.	2
Atheta (*Microdota*) *Spatula* Fauv.	.	.	.	1	.	.	.	.	.	.
Oxypoda haemorrhoea Man.	.	1	.	.	.	.	.	.	.	.
Oxytelus rugosus F.	.	.	1	.	.	.	.	.	.	.
Quedius fulgidus F.	.	1	.	.	.	.	.	.	.	.
Sipalia circellaris Gr.	.	.	.	4	.	.	1	1	.	1
Trechus minutus F. (=*quadristriatus* Schr.)	.	.	1	1	.	.	.	.	.	.
Woodlice (Isopoda):										
Species not identified	.	×	.	.	.	.	.	.	.	.

The asterisk under nest 58 indicates the presence of one flea which was lost and therefore not identified. It has been thought worth while to tabulate the results in such a way that future workers can add to them and so gradually work out correlations between different species. It is not clear, for instance, what foods are eaten by the predatory beetles; probably they depend on flea larvae or Collembola. Such beetles may play a part in keeping down flea numbers. Miss Hora records 12 species of mites, of which she states three to be probably accidental visitors. It is not known how many beetles and Collembola come also in this category: but it is not safe to assume that a species is an accidental visitor to mouse nests simply on the ground that it occurs commonly outside them, although this is undoubtedly the case with certain species. It will be seen that *Ctenophthalmus agyrtes celticus* was by far the most abundant flea, while *Typhloceras poppei* was very rare and *Ceratophyllus fasciatus* absent. The adult flea population ranged from 1 to 64, the mean (of nests containing any fleas at all) being about 15. The absence of *C. fasciatus* is striking, since Brown Rats are numerous in the villages of the west coast of Lewis. The total number of species actually recorded from these Lewis House Mouse nests is 30, which is a figure similar to those obtained from complete surveys of mammal and bird host populations, *e.g.* on *Apodemus*

sylvaticus at Oxford, 47; on Marmots (*Citellus pygmaeus*) in South-east Russia, 50; on Brown Rats (*Epimys norvegicus*) from various parts of England, 27 (for references see Elton, 1933, p. 22); on the Bobwhite Quail (*Colinus virginianus*) in Southern Georgia and Northern Florida, 36 (Stoddard, 1932); and on the Red Grouse (*Lagopus scoticus*) in various parts of Scotland, 22 (Leslie and Shipley, 1912). In all these instances the number of species of parasites or nest inhabitants was more than 20 and less than 51. We know as yet little of the inter-relations of the members of these parasite communities, except that there is a well-marked specialised distribution in different regions of the body of the host.

IV. Discussion

Fleas. Ctenophthalmus agyrtes celticus and *Typhloceras poppei* occurred both on *Apodemus* and on *Mus*, the former being relatively common and the latter rare. *Ceratophyllus fasciatus* occurred on two rats, and was rare on *Mus* and absent from *Apodemus*. *Ctenophthalmus agyrtes celticus* was the commonest flea found both on *Apodemus* and voles (*Evotomys* and *Microtus*) near Oxford (Elton and others, 1931). It also formed half the flea population on rats, *Epimys norvegicus*, living in East Anglian fields (Martin and Rowland, 1911, p. 44). It was also the commonest flea taken on *Mus* at Ollerberry in the Shetlands, where it also occurs on *Apodemus* (Waterston 1914). It also occurs on *Mus* in the Orkneys (Waterston). *Typhloceras poppei* is stated by Rothschild (1915) to occur in England and Scotland, the usual host being *Apodemus sylvaticus*. Waterston found one in a nest of *Mus* at Ollerberry, and 48 in a nest of *Apodemus* at the same place. It is also found in Ireland, together with *Ctenophthalmus agyrtes celticus*. *Ceratophyllus fasciatus* is the common rat flea in Great Britain, but is evidently not common on *Mus* in Lewis. It was found by Harrisson and Moy-Thomas (1933) on *Mus musculus* in St Kilda, and occurred very occasionally upon *Apodemus sylvaticus* in Bagley Wood, Oxford. It also formed half the flea population on Brown Rats, *Epimys norvegicus*, living in fields in East Anglia. The absence of the common vole flea, *Ceratophyllus penicilliger* Grube, from these Lewis collections is probably significant. This species occurred on *Apodemus*, *Evotomys*, and *Microtus* at Oxford, but was only common on *Evotomys*. It also occurs on voles in Lapland (Elton, 1934), so that its absence from northern islands of Great Britain is unlikely to be due to climatic reasons. Waterston does not record it from Shetland, but states that it occurs on the Orkney vole (*Microtus orcadensis* Millais). It is absent from Ireland. No voles occur in Shetland, Lewis, or Ireland, so that *Ceratophyllus penicilliger* is evidently a later immigrant with voles, to which it must be primarily attached, while straying on to other small mammals. Judging by the facts given already, *Typhloceras poppei* is primarily an *Apodemus* flea, and is very unlikely to have been introduced into Lewis, Shetland or Ireland on the House Mouse. We may therefore conclude that it probably entered the islands with the original

population of *Apodemus*. The same may be true of *Ctenophthalmus agyrtes celticus*, but its common occurrence on rats and House Mice complicates matters. Thus the House Mice examined on St Kilda by Harrisson and Moy-Thomas were practically all caught in the houses, not out-of-doors. (They examined very few *Apodemus* for parasites: Moy-Thomas, personal information.) *Ceratophyllus fasciatus* is obviously a later immigrant on rats or House Mice. The two nematode parasites found by me in *Apodemus* in Lewis both occur in the Oxford district. The *Syphacia* known in rats and House Mice may be a different species from that in *Apodemus* (see footnote, p. 108). *Nematospiroides dubius* is entirely confined to *Apodemus*, so far as is known, and was never found in voles at Oxford. It will be noted that there is great overlapping of the flea-host relations, between *Apodemus*, *Mus*, and *Epimys*, on Lewis. This phenomenon was pointed out for Oxford rodents and shrews and moles (Elton and others, 1931), and also occurs very markedly among small mammals in Lapland (Elton, 1934). It is of importance in view of the possible spread of epidemic diseases—now proved to be of common occurrence among small mammals in nature—from one species of host to another, by means of blood-sucking or other ectoparasites. Of significance here is Miss Hora's observation that several of the species of mites are blood-suckers, and that these also often occur on more than one species of mouse.

V. SUMMARY

A preliminary survey of parasites from *Apodemus hebridensis*, *Mus musculus* and *Epimys norvegicus* on the Isle of Lewis, Outer Hebrides, is described; also the fauna of House Mouse nests. Special discussion is given of the flea fauna, and it is shown that there is much overlapping in host relations. The original flea fauna of *Apodemus* on the outer islands of the British Isles is indicated.

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A THIRD LIST OF PARASITIC COPEPODA OF PLYMOUTH WITH NOTES

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(With 2 Figures in the Text)

The following is supplementary to the previous lists (Leigh-Sharpe, 1926 and 1933), and consists of records of parasitic Copepoda taken by workers in that group who have visited the Laboratory of the Marine Biological Association, Plymouth, during 1933.

The names of collectors given in the list are abbreviated as follows: G. = Dr R. Gurney (July); L. = W. H. Leigh-Sharpe (June, Aug.–Sept.); O. = Dr C. L. Oakley (June).

Parasite	Author	Collector	Location	Host	See Note
		LICHOMOLGIDAE			
Modiolicola inermis	Canu	L.	Mantle cavity	*Pecten maximus*	—
		CALIGIDAE			
Caligus pelamydis	Krøyer	L.	Gills	*Pelamys sarda*	B
Pseudocaligus brevipedes	(Bassett-Smith)	L.	Side of head	*Gobius paganellus*	A, C
Pandarus bicolor	Leach	O.	Middle of side of body	*Squalus acanthias*	A, D
Nogaus ambiguus	T. Scott	O.	Side of body	„ „	—
		DICHELESTHIIDAE			
Hatschekia labracis	(van Beneden)	G.	Gills	*Labrus berggylta*	—
		LERNAEIDAE			
Lernaeocera branchialis	Linnaeus	L.	Gills	*Merluccius merluccius*	A
L. lusci	(Bassett-Smith)	L.	Gills	*Molva molva*	A
		CHONDRACANTHIDAE			
Chondracanthus merluccii	(Holten)	L.	Gills	*Gadus luscus*	A
		LERNAEOPODIDAE			
Lernaeopoda bidiscalis	Kane	L.	Apex of clasper	*Galeorhinus galeus*	E
Clavella deliciosa	Leigh-Sharpe	L.	Gills	*Pollachius virens*	A

Notes on species in the list

A New host in addition to previous records.

B New host for British waters, taken in St Austell Bay.

C Taken at Wembury, where the goby would be in association with rockling, the usual host. Infection probably accidental.

D Of about a hundred examined in June, about eighty bore on an average two females and one male. On about four hundred examined in September no parasites were found.

E The parasite, which lacked eggstrings and one abdominal appendage, had the cephalothorax and most of the trunk deeply embedded in a cavity which it had eroded in the apex of the right clasper of the tope caught between Looe and Rame Head, 2. ix. 33, by a local fisherman (Fig. 1). As I suspected, Kane is wrong in stating that *Lernaeopodia bidiscalis* has no bulla, since a careful dissection reveals that it possesses a small vestigial one like that of *L. globosa* Leigh-Sharpe (1918) (Fig. 2).

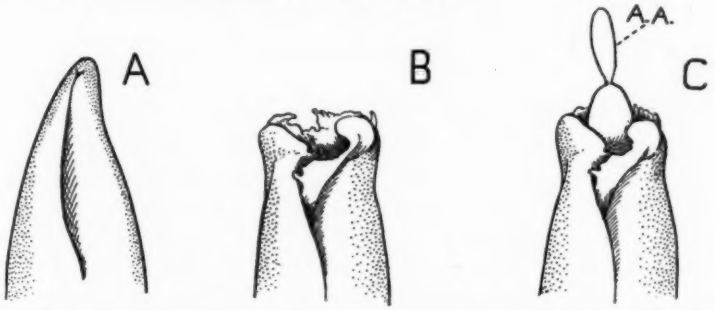

Fig. 1. *Galeorhinus galeus*. A, apex of left clasper, normal. B, Apex of right clasper showing erosion made by *Lernaeopoda bidiscalis*. C, Apex of right clasper with *L. bidiscalis in situ* *A.A.* = abdominal appendage.

Fig. 2. *Lernaeopoda bidiscalis* ♀. Enlarged end of a second maxilla. *B* = bulla.

ADDITIONAL NOTES ON SPECIES PREVIOUSLY RECORDED

(1) *Lernaeocera phycidis* Leigh-Sharpe, of which last year I had only the one type specimen, appeared much more common in September 1933, as I obtained four specimens on about thirty *Urophycis blennoides* examined from Milford Haven.

(2) *Chondracanthus zei*, usually so common, seemed rare in September, as I found none on twenty *Zeus faber* examined, both large from Milford Haven and small from Plymouth. Reported common earlier in the year.

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A LIST OF PARASITIC RHABDOCOELA (TURBELLARIA) OF PLYMOUTH

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(With 2 Figures in the Text)

No worker on British Turbellaria having hitherto given any attention to parasitic species, I devoted the greater part of my visit to the Laboratory of the Marine Biological Association, Plymouth, Aug.–Sept. 1933, to the collection and investigation of these forms. The number discovered is small in proportion to the number of hosts examined. All but the last have been identified from von Graaf (1913), and all but the first and last are new to the British fauna.

Sub-order RHABDOCOELA

Parasite	Author	Location	Host	See Note
		ANOPLODIIDAE		
Syndesmis echinorum	François 1881	Intestine	*Echinus esculentus*	A
Anoplodium parasitica	Schneider 1858	Body cavity	*Holothuria forskali*	B
Umagilla forskalensis	Wahl 1906	Fore-part of intestine	„ „	C
		GENOSTOMATIDAE		
Genostoma tergestinum	(Calandruccio) 1884	Under gill cover	*Nebalia bipes*	D
Genostoma marsiliense	(Calandruccio) 1888	Under gill cover	„ „	D
		GRAFFILLIDAE		
Paravortex cardii	(Hallez) 1908	Ovary	*Cardium edule*	E
Graffilla gemellipara	Linton 1910	Surface of body Testes	„ „	E

Notes on species in the list

A Of the three *Echinus* examined eight parasites were found in the first, one in the second, a half-grown urchin, on 1. ix. 33, and fourteen in the third on 5. ix. 33.

B Two specimens in the only large *Holothuria* examined, 2. ix. 33. Two small *Holothuria* were without parasites.

C One specimen in the same *Holothuria*. The third species, *Anoplodium gracile* Wahl 1906, was not found.

D Of three *Nebalia* from Rum Bay on 4. ix. 33 one was infected with six *Genostoma*, three of each species.

E One of each species in different *Cardium* from Niele Point, in a dozen examined.

The two species can be distinguished as follows:

Paravortex cardii	*Graffilla gemellipara*
Length: Up to 1 mm.	Up to 2 mm.
Swims in circles	Creeps
No excretory bladders	Paired, anterior excretory bladders
Ovary not long, palmate	Ovary abnormally elongated
Embryos pass out and mother lives on	Gonads disintegrate, mother dies and embryos are liberated

All but the last distinction have been verified by the author.

As Linton (1910) has not figured the reproductive systems of *Graffilla gemellipara* I have drawn the details from a compressed specimen stained with Delafield's haematoxylin. (Figs. 1 and 2.)

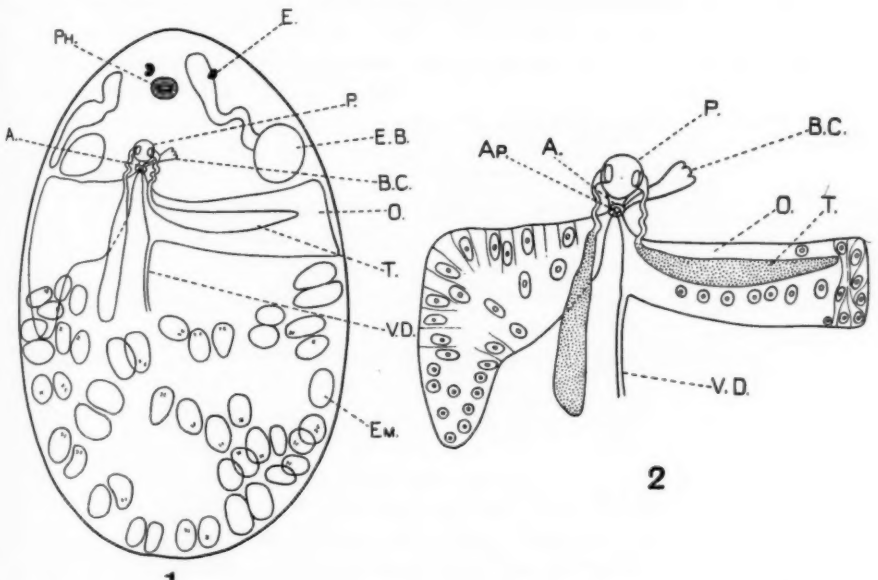

Fig. 1. *Graffilla gemellipara*. *A.* = atrium; *B.C.* = bursa copulatrix; *E.* = eye; *E.B.* = excretory bladder; *Em.* = embryos; *O.* = ovary; *P.* = penis; *Ph.* = pharynx; *T.* = testis; *V.D.* = vitelline duct.

Fig. 2. *Graffilla gemellipara*. Enlarged view of the reproductive systems, lettering as in Fig. 1. *AP.* = common genital aperture.

The following animals were examined without, so far, success as regards revealing parasites, those marked * being infected in the Mediterranean and elsewhere: Various anemones (12) (*Actinia, Cerianthus, Sargartia*) (coelenteric cavity); *Porania* (1), *Cucumaria* (2), *Echinocardium** (5), *Thalassema* (1) (body cavity and intestine); *Mytilus** (10), *Tellina crassa** (8), *Paphia* [*Tapes*]* (8), *Solen** (1) (gills, mantle-cavity, stomach and intestine); *Buccinum** (2), *Ocenebra* [*Murex*]* (1) (liver, kidney, gonad, intestine); *Astacus homarus* (1) (gills).

My best thanks are due to Mr W. H. Leigh-Sharpe for his kind assistance.

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ON A TREMATODE *ALLOPYGE UNDULATUS* N.SP. PARASITIC IN LILFORD'S CRANE (*MEGALORNIS GRUS LILFORDI*)

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(With 1 Figure in the Text)

While studying a collection of roundworm parasites from animals dying in the Philadelphia Zoological Garden, accounts of which appeared in 1929 and 1931, I found ten flukes in it, one of which proved to be new. Two common distomes in the lot, *Renifer ellipticus* Pratt 1903 and *Opisthorchis tenuicollis* (Rud. 1819) are from new hosts; the former from an American beaver (*Castor canadensis*) and the latter from a Canadian lynx (*Felis canadensis*). The one rare monostome found in the lot is recognised as a new species of the genus *Allopyge* Johnston 1913 in the family Cyclocoelidae and is named *Allopyge undulatus* n.sp. It was found in the thoracic air sacs of Lilford's crane (*Megalornis grus lilfordi*).

The following is a list of the Trematodes, arranged according to hosts:

Mammalia.

Trichechus manatus (West Indian manatee).
Chiorchis fabaceus (Dies. 1838). Colon.
Felis canadensis (Canadian lynx).
Opisthorchis tenuicollis (Rud. 1819). Bile duct.
Didelphys virginiana (common opossum).
Harmostomum opisthotrias (Lutz 1895). Ileum.
Rhopalias macracanthus Chandler 1932. Small intestine.
Castor canadensis (American beaver).
Renifer ellipticus Pratt 1903. Caecum.

Reptilia.

Natrix sipedon (brown water-snake).
Pneumatophilus variabilis (Pratt 1903). Trachea.
Dasymetra conferta Nicoll 1911. Pharynx.
Lechriorchis elongatus (Pratt 1903). Oviduct.

Aves.

Sarcorhamphus papa (King Vulture).
Paryphostomum segregatum Dietz 1909. Intestine.
Megalornis grus lilfordi (Lilford's crane).
Allopyge undulatus n.sp. Thoracic air sacs.

Family CYCLOCOELIDAE Kossack

Allopyge undulatus n.sp. (Fig. 1)

The Cyclocoelidae has been divided into from six to sixteen genera in the past. They were recently reduced to three by Joyeux and Baer (1927). I am of the opinion, however, that this cannot be accepted in view of the fact that

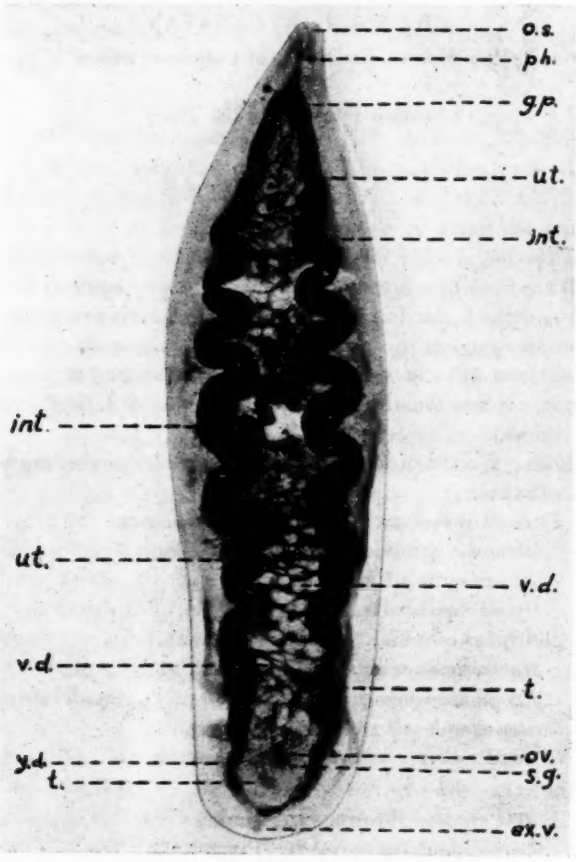

Fig. 1. Photomicrograph of *Allopyge undulatus* n.sp. Ventral view. ×11.

Lettering. *ex.v.* excretory vesicle; *g.p.* genital pore; *int.* intestine; *o.s.* oral sucker; *ov.* ovary; *ph.* pharynx; *s.g.* shell gland; *t.* testis; *ut.* uterus; *v.d.* vas deferens; *y.d.* yolk duct.

some important generic characters, namely, the position of the genital pore, the character of the uterus and the character of the intestinal limbs, vary more than they allow for in their generic diagnoses. I am prompted to advance

this opinion because I have a specimen from Lilford's crane (*Megalornis grus lilfordi*) that does not readily fit into any description given by the above authors for their three genera although it is a member of the family Cyclocoelidae. It readily fits into the genus *Allopyge* Johnston 1913 and is closely related to *A. antigones* Johnston 1913 from the great Australian crane (*Antigones australasiana*). It has all of the characteristics of that species with the following exceptions: the size of the worm, the marked difference in the character of the uterus, and it is from a different host species.

This new species is 11–11·5 mm. long by 2·75–3 mm. broad. *Allopyge antigones* is much larger, being 20 × 4 mm. It is about the size of *Hyptiasmus adolphi* Stossich 1902 found in several species of European cranes (Gruiformes). *H. adolphi*, however, has its genital pore anterior to the pharynx while this new one has its genital pore behind the intestinal fork as in *Allopyge antigones*. The uterus of this new one differs from the uterus of *A. antigones* in that it is greater in diameter throughout and its fewer loops overlap upon one another. A very prominent feature is the wavy appearance of its intestinal limbs and it is because of this that I propose to call it *A. undulatus* n.sp.

Briefly, the body tapers at both ends, more sharply at the anterior. Character of oral sucker and pharynx as in all members of the family. Short oesophagus divides into undulating intestinal limbs lying approximately midway between sides and mid-line of body, joined at hinder end. Uterine loops confined to space between intestinal limbs. No backwardly directed loops extending behind posterior testis. Anterior testis round and lateral to mid-line; posterior testis oval and transversely placed with vas deferens arising from antero-lateral border. Vasa deferentia run anteriorly and join into one duct some distance behind cirrus sac. Small ovary between testes nearer posterior testis and separated from the latter by a number of uterine loops. Ovary and testes in a straight line inclined at an angle to median line and within posterior eighth of body. Yolk duct arches its way across intertesticular space just posterior to ovary. Excretory system with dorsal and ventral network and two main dorsal trunks. The position of the vesicle and pore as in other members of the genus. Laurer's canal absent; receptaculum seminis present. Eggs not very numerous, yellow to light brown *in utero*.

Type specimen slide No. 174 (1) P.Z.G. 4991 on deposit in the Zoological Department of the University of Pennsylvania.

Host: Megalornis grus lilfordi (Lilford's crane). Thoracic air sacs, 10 specimens; distribution, Eastern Siberia to North-west India.

Two additional Trematodes that have been identified are appended here:

DIGENEA

Renifer ellipticus Pratt 1903

Host: Agkistrodon piscivorus (water moccasin). Mouth.

Collector: Dr Roger Conant, director of the Toledo (Ohio) Zoological Society's Garden.

MONOGENEA

Epibdella bumpusii (Linton)

Host: A member of the Gadidae. Gills.

Collector: Dr Henry W. Fowler, ichthyologist of the Philadelphia Academy of Natural Sciences. Collected in Labrador.

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ON *COITOCAECUM ANASPIDIS* SP.NOV., A TREMATODE EXHIBITING PROGENESIS IN THE FRESH-WATER CRUSTACEAN *ANASPIDES TASMANIAE* THOMSON

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(With 6 Figures in the Text)

The recorded instances of a Trematode attaining a state of maturity in an invertebrate host are comparatively few. Sinizin (1905) drew attention to the fact that *Pleurogenes medians* (Olsson) is often found as a metacercaria in the aquatic larva of *Agrion* sp., and that while in this condition it sometimes exhibits a state of precocious genital activity giving rise to eggs in the uterus. Dollfus (1924) doubts the viability of these eggs observed by Sinizin, but records that *Pleurogenes medians* shows a similar state of advanced development whilst encysted as a metacercaria in *Gammarus pulex* (L.). Fuhrmann (1928) mentions other instances of progenesis among the Trematoda and points out that in the case of *Ratzia parva* Poche, which is found encysted in the muscles of the frog, the eggs in the uterus of the metacercaria contain a well-developed miracidium. The fresh-water crayfish, *Potamobius fluviatilis* (Fabricius), plays the part of a secondary host to *Astacotrema cirrigerum* (von Baer), which develops precociously in the muscles of the decapod.

In addition to the above instances of apparently abnormal development while in an encysted condition, we have several examples of Trematodes which develop quite normally in an invertebrate as definitive host, namely *Aspidogaster conchicola* von Baer, in various species of *Unio*, and *Cleistogamia holothuriana* Faust (1927) in the holothurian, *Actinopyga mauritiana*.

The present paper deals with a further example of progenesis exhibited by a Trematode found encysted in *Anaspides tasmaniae* Thomson. This fresh-water shrimp is known to occur only in a few tarns and streams on the mountains of Tasmania. In the New Town Creek and other small streams on Mt Wellington the shrimp is infected with an encysted Trematode belonging to the genus *Coitocaecum* Nicoll (1915) in the family Coitocaecidae Ozaki (1929).

The Trematode proves to be a new species and the name *Coitocaecum anaspidis* is suggested. A systematic description of the species is given below.

The cysts

The cysts are not confined to any one part of the shrimp. The hepatic caeca, reproductive organs, body muscles and the larger blood sinuses are often infected by the encysted metacercariae in various stages of development. More

frequently than elsewhere the cysts occur in the ventral sinus, especially in the last two abdominal segments. Smaller cysts sometimes occur in the basal joints of the legs and pleopods. They can often be seen through the more or less transparent exoskeleton of the living shrimp. The cysts do not occur in the alimentary canal. The only parasite observed in the digestive tract was the interesting gregarine, *Ganymedes anaspidis* J. Huxley (1910).

The cysts of *Coitocaecum anaspidis* are approximately oval. They vary in size according to the degree of development of the enclosed metacercaria. Three of the largest cysts had the following measurements in mm.: 1·16 × 0·698, 1·16 × 0·812, and 0·986 × 0·638 respectively. The wall of the cyst is usually colourless and transparent, but in some specimens it is yellowish brown. It is elastic and readily deformed by the active movements of the enclosed Trematode, which twists about in the colourless fluid inside the cyst.

In order to determine the degree of infection of the shrimp, 200 specimens were collected during July and August, 1932, from the New Town Creek. Each one was carefully dissected under the binocular microscope and the cysts counted. Only 73 shrimps were free from cysts. The remaining 127 specimens contained a total of 321 cysts, the largest number in one shrimp being 24.

A fish is the definitive host of all known Coitocaecidae, but in the small streams on the slopes of Mt Wellington there are no fish and *C. anaspidis* appears to complete its life history as a progenetic metacercaria in the shrimp. In 201 out of 321 cysts the Trematode had actually laid eggs, which in some cases were so numerous as almost to hide the body of the parasite (Fig. 3). These eggs often contain a well-developed miracidium, which can be seen moving inside the shell. In 20 cysts some of the eggs had actually hatched and the ciliated miracidia were observed swimming about actively in the clear fluid of the cyst.

When a cyst contains a fully developed Trematode surrounded by a large number of eggs, it readily bursts. This seems to occur quite frequently inside the shrimp, with the result that the eggs and any free miracidia escape into the blood sinuses and are carried to various parts of the body. Specimens of *Anaspides tasmaniae* are often found in which the eggs of the Trematode have been lodged in the epipodial lamellae in the above manner. This observation was confirmed as follows: a shrimp, in which a cyst could be seen near the surface of the last abdominal segment, was taken and gentle pressure applied near the cyst. This caused the cyst to burst without injuring the shrimp. The liberated eggs were immediately carried forward by the blood stream in the ventral sinus and lodged in the pleopods, legs and epipodial lamellae. The shrimp, which seemed to suffer no immediate ill effects from this treatment, was placed in an aquarium where it lived for seven days.

When a cyst bursts the Trematode seems unable to encyst itself again. It makes its way through the ruptured wall of the cyst and for a short time moves about inside the shrimp. However, it soon dies, becoming hard and shrunken.

These dead Trematodes appear to undergo partial absorption in the fluids of the host's body.

The eggs, which are carried into the epipodial lamellae, generally remain there and cause stoppage of the blood stream accompanied by disintegration of the appendage concerned. This allows the eggs to fall into the water. If a cyst should burst without all the eggs escaping, it ultimately becomes a hard, dark brown mass consisting of the wall of the cyst and the remaining eggs cemented together. This mass often surrounds the dead body of the Trematode and contains many open egg-shells, from which the miracidia have already escaped.

EGGS AND MIRACIDIA

The eggs are golden brown and oval. They measure 0·074–0·083 × 0·046–0·049 mm. The egg-shell is modified at one end to form an operculum, which has a diameter of 0·018 mm. (Fig. 5). Some eggs containing active miracidia were placed in water from the creek and the method of hatching was observed under the microscope. The miracidium rotates with the anterior end of its body pressed against the operculum. After an hour or longer it succeeds in escaping, the operculum being raised as if on a hinge. The vacated shell, which still contains a few yolk granules, sometimes adheres to the long cilia at the posterior end of the miracidium and is carried through the water as the ciliated larva moves about in search of an intermediate host.

When at rest the miracidium is oval and measures 0·064–0·083 × 0·037–0·055 mm. When moving it becomes somewhat pyriform, and two slight annular constrictions divide its body into three parts (Fig. 2). The whole body is clothed with cilia which increase in length towards the posterior end. The ciliated larva moves with the narrow end directed towards the front and slowly rotates as it makes its way through the water. Two conspicuous germinal bodies occur one on each side of the posterior third of the miracidium, and a rudimentary enteron, containing yolk granules, occupies the anterior half. On each side of the enteron is a flame cell, which appears to open directly on the exterior. No eye spot is visible.

OTHER HOSTS

In addition to *Anaspides tasmaniae*, a species of *Gammarus*, which occurs in the same creeks, is infected by the metacercaria of *Coitocaecum anaspidis*. Out of twenty-five specimens of *Gammarus* sp. examined, seven were found to contain cysts, the total number of cysts being eleven. In five cases the Trematode had laid eggs in the cysts, and in two cases the cysts contained free-swimming miracidia as well as eggs.

The fresh-water crayfish, *Astacopsis franklinii* Gray, which is also found in the creek, does not appear to be attacked by the Trematode.

The aquatic larvae of insects belonging to the genera *Atalophlebia* (Plectoptera), *Eusthenia* (Perlaria), *Aeschna* (Odonata) and *Helicopsyche* (Trichoptera),

occur in association with *Anaspides tasmaniae*, but are not infected by the Trematode.

Fresh-water snails are very rare at the locality. After a search extending over six months, during which time a large quantity of debris from the creek was examined, only two small Gastropods were found. When these were placed in water containing the miracidia of *Coitocaecum anaspidis*, the latter made no attempt to penetrate them. The high degree of infection suffered by *Anaspides tasmaniae* would imply the presence of a fairly plentiful intermediate host, and the evidence points to this host not being a fresh-water snail.

Systematic description of *Coitocaecum anaspidis*

External characters. In life *C. anaspidis* is almost cylindrical, with the extremities somewhat narrowed and bluntly rounded. When removed from a cyst and placed in normal saline the Trematode is able to extend itself to almost 3·0 mm. in length. It is very active and constantly changing in shape, one moment being leaf-like, the next elongated and vermiform. The part of the body in front of the acetabulum is neck-like when fully extended. After fixation specimens measure 2·4–2·8 × 0·75–0·76 mm.

The surface of the body is smooth and has a white translucent appearance. The testes, ovary, vitelline glands and eggs are clearly visible through the cuticle. The oral sucker is subterminal and measures 0·164 mm. in diameter. The acetabulum is deep, cup-like and about two-fifths of the body length from the anterior end. It measures 0·37 × 0·26 mm.

Alimentary system. The oral sucker leads into a very short prepharynx, which is followed by a globular pharynx, 0·082 mm. in diameter. A thick-walled oesophagus, 0·153 mm. long, passes back to join the intestine, the two limbs of which run out to the sides and follow a straight course to within 0·307 mm. from the end of the body. Here the two rami bend round and unite with each other below the excretory vesicle (Fig. 1). The intestine is simple in form, showing no signs of lateral caeca. There is no anal aperture.

Excretory system. The excretory vesicle is fusiform and very large, resembling that described by Johnston (1912) in the case of *Dolichosaccus ischyrus*. It extends forwards as far as the ovary. Near its anterior end it gives off a pair of fine collecting vessels, which run out to the sides, follow a course parallel with the limbs of the intestine and extend to the level of the pharynx. These collecting ducts are connected with a network of tubules near the surface of the body. The vesicle opens on the exterior by an excretory pore at the posterior end of the body.

Muscular system. The muscles of the body wall are arranged in the usual three layers, namely, an outer layer of circular muscles, an inner layer of diagonal muscles, and between the two a layer of longitudinal muscles. Near the sides of the body and round the ventral sucker are a number of dorso-ventral muscle fibres directed perpendicularly or obliquely.

Nervous system. The central nervous system consists of a pair of large

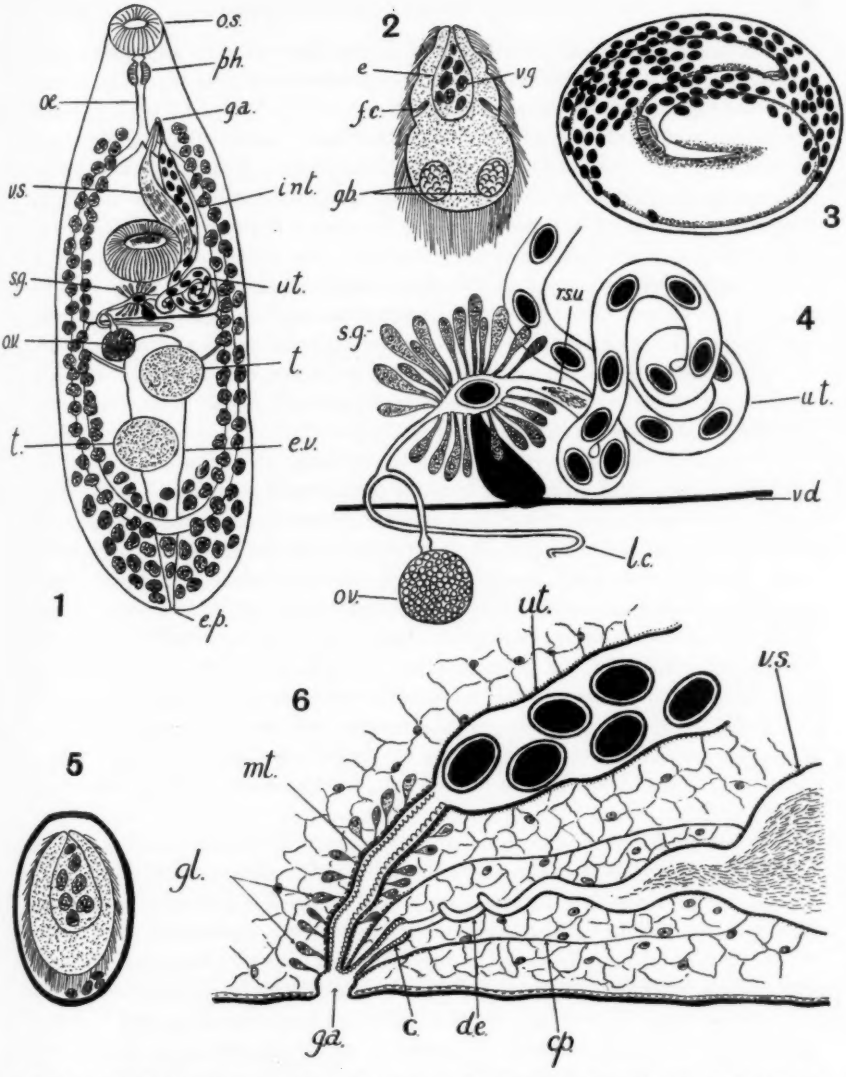

Fig. 1. Ventral view: *e.v.* excretory vesicle; *e.p.* excretory pore; *g.a.* genital aperture; *int.* intestine; *oe.* oesophagus; *o.s.* oral sucker; *ov.* ovary; *ph.* pharynx; *s.g.* shell gland; *t.* testes; *ut.* uterus; *v.s.* vesicula seminalis.

Fig. 2. Miracidium: *e.* enteron; *f.c.* flame cell; *g.b.* germinal bodies; *v.g.* vitelline granules.

Fig. 3. Cyst containing the Trematode and eggs.

Fig. 4. Ventral view of female genital complex: *l.c.* Laurer's canal; *ov.* ovary; *r.s.u.* receptaculum seminis uterinum; *s.g.* shell gland; *v.d.* transverse yolk duct; *ut.* uterus.

Fig. 5. Egg containing miracidium and yolk granules.

Fig. 6. Male and female end-organs: *c.* cirrus; *c.p.* cirrus pouch; *d.e.* ductus ejaculatorius; *g.a.* genital aperture; *gl.* glands opening into metraterm; *mt.* metraterm; *ut.* uterus; *v.s.* vesicula seminalis.

cerebral ganglia situated dorso-laterally to the pharynx and connected by a thick commissure, which crosses above the middle of the pharynx. From each cerebral ganglion three nerves are given off anteriorly. Two of these pass to the oral sucker and the third goes to the side of the body, where it bends round and forms a lateral nerve running towards the posterior end. From the inner side of each cerebral ganglion a short thick nerve passes directly to the pharynx. Posteriorly each ganglion gradually merges into a thick longitudinal nerve cord, which runs outwards and downwards to the ventral side, following a course below the corresponding intestinal ramus. These two principal nerve trunks pass one on each side of the acetabulum, which receives a number of small branches from them. The main cords pass almost to the end of the body.

Reproductive system. The genital system opens by the genital pore, which is on the ventral surface to the left and slightly in front of the point where the oesophagus joins the intestine.

The ovary is almost spherical, and about 0·09 mm. in diameter. It is on the right side, below and slightly in front of the excretory vesicle. The oviduct leaves the ovary on the antero-dorsal side and its initial part is slightly enlarged to form a fertilisation chamber. It then passes under the transverse yolk duct, turns upward to the right, and gives off the Laurer's canal. This canal bends posteriorly and then crosses the body transversely to open on the dorsal surface, immediately above the anterior end of the excretory vesicle and to the left of the middle line. A short distance beyond its junction with Laurer's canal the oviduct passes into the ootype. Here it receives the ducts of the shell gland and is connected directly with the median yolk reservoir. The cells of the shell gland are arranged radially round the ootype, but are not closely aggregated. Their ducts are long and slender. A true receptaculum seminis is absent (Fig. 4).

The convolutions of the uterus are intercaecal and made up of only two or three loops, which, in specimens with a small number of eggs, lie mainly on the left side between the anterior testis and acetabulum. When the uterus contains a large number of eggs its loops extend across the body but do not overlap the intestinal rami. The uterine wall is very thin and consists of an inner layer of longitudinal muscles and an outer layer of circular muscles. The initial part of the uterus, near the ootype, is often filled with spermatozoa and forms a receptaculum seminis uterinum. The anterior part of the uterus passes over the left side of the acetabulum and runs forward in a straight course near the dorsal surface of the body. Finally it passes under the left intestinal ramus near the oesophagus and ends in a well-developed metraterm, which opens at the genital pore. The wall of the metraterm consists of an inner layer of strong longitudinal muscles and an outer layer of circular muscles, while its lumen is lined with an epithelium, the cells of which form papillae. The length of the metraterm is about 0·122 mm. and it is perforated by the ducts of a number of unicellular glands lying in the surrounding parenchyma (Fig. 6).

The vitelline glands consist of numerous oval follicles, which have an

average size of 0·061 × 0·049 mm. They lie mainly above, below and on the outer side of the intestinal rami. At the posterior end of the body and occasionally along the sides they surround the intestine. There is a longitudinal yolk duct on each side running close to the intestine and receiving small vessels from the follicles. The anterior and posterior portions of the longitudinal ducts unite at the level of the anterior edge of the ovary. From the point of union on each side a transverse duct leads to the base of a triangular yolk reservoir, which in most specimens is in the middle line of the body, but in some is displaced to the right. The vitelline glands extend from the posterior end of the body to the level of the genital aperture.

The two testes are large ovoid bodies, about 0·20 × 0·15 mm. They lie almost one behind the other immediately below the anterior half of the excretory vesicle. The two vasa deferentia proceed in a straight course from the testes to the base of the vesicula seminalis, where they unite. The vesicula seminalis is an elongated sac measuring 0·30 × 0·06 mm. It lies above the left side of the acetabulum and below the uterus. With the exception of its narrow anterior end, it is wholly outside the cirrus pouch (Fig. 6). The wall of the vesicula seminalis consists of an outer layer of very delicate circular muscles and an inner layer of faintly marked longitudinal fibres. The cirrus pouch is about 0·20 mm. long and contains a spirally twisted ductus ejaculatorius ending in an unarmed cirrus. A definite pars prostatica appears to be absent and there is no distinct prostate gland. The cirrus is about 0·063 mm. long. Its lumen is lined with an epithelium similar to that lining the metraterm and its wall is thick and muscular. The anterior portion of the cirrus pouch, or the part which immediately surrounds the cirrus, is bounded by a wall consisting of inner longitudinal muscles and outer circular muscles. Posteriorly these muscles disappear, so that the portion of the cirrus pouch surrounding the ductus ejaculatorius is bounded by a wall of flat epithelial cells containing large nuclei. This wall is continued back so as to enclose the tapering anterior end of the vesicula seminalis. The cirrus leads to the genital pore.

Affinities. *Coitocaecum anaspidis* seems to be more closely related to *C. unibulbosum* Ozaki than to any other member of the genus. It may be distinguished from this and other species by means of the following table, which is based partly on one by Ozaki (1929):

1.	Vitellaria entirely postacetabular	C. gymnophallum Nicoll.
	Vitellaria extend into neck 2	
2.	Body round	C. latum Ozaki.
	Body elongated oval 3	
3.	Pharynx constricted	C. diplobulbosum Ozaki.
	Pharynx globular 4	
4.	Wall of cirrus pouch muscular throughout its length	C. plagiorchis Ozaki.
	Wall of cirrus pouch partly or wholly membranous 5	
5.	Vesicula seminalis externa globular	C. orthorchis Osaki.
	Vesicula seminalis externa tubular 6	
6.	Oesophagus short. Cirrus absent	C. unibulbosum Ozaki.
	Oesophagus long. Cirrus present	C. anaspidis sp.nov.

SUMMARY

1. *Coitocaecum anaspidis* sp.nov. is a Trematode which exhibits precocious genital activity whilst encysted as a metacercaria in the Tasmanian Mountain Shrimp, *Anaspides tasmaniae* Thomson.

2. Eggs are laid by the Trematode whilst in the encysted condition, and in some cases the eggs hatch in the cyst giving rise to miracidia which swim freely in the contained fluids.

3. Two hundred specimens of *Anaspides tasmaniae* were examined and one hundred and twenty-seven found to contain the encysted Trematode.

4. A systematic description of the Trematode is given.

ACKNOWLEDGMENT

I desire to express my gratitude to Mr F. J. Brown for much kindly help and for literature not readily accessible in Tasmania.

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SOME PARASITIC WORMS FROM AUSTRALIA

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(With 3 Figures in the Text)

In this paper short descriptions are given of three new species (two Cestodes and a Nematode) contained in a collection kindly entrusted to the writer by Prof. G. H. F. Nuttall, F.R.S., for determination. The material was collected in Queensland by Mr F. H. S. Roberts, and forwarded to Prof. Nuttall by Mr Robert Veitch, Chief Entomologist, Department of Agriculture and Stock, Brisbane.

In addition to the three new forms to be described below, the collection included the following species:

CESTODES

Fam. ANOPLOCEPHALIDAE.

Prototaenia ?obesa (Zschokke 1898)—intestine of "native bear" (*Phascolarctos cinereus*), Brisbane.

Fam. DAVAINEIDAE.

Raillietina (*Paroniella*) *corvina* (Fuhrmann 1905)—intestine of hazel-eyed crow (*Corvus cecilae*), Burketown.

Fam. HYMENOLEPIDIDAE.

Diorchis flavescens (Krefft 1871)—intestine of grey teal (*Querquedula gibberifrons*), Burketown.

NEMATODES

Fam. HETERAKIDAE.

Heterakis gallinae (Gmel. 1790).

H. isolonche v. Linst. 1906.

These two species occurred together in the caeca of an Amherst pheasant (*Chrysolophus amherstiae*) at Brisbane.

Fam. STRONGYLIDAE.

Labiostrongylus labiostrongylus Yorke and Maplestone 1926.

Macropostrongylus macropostrongylus Yorke and Maplestone 1926.

M. australis Yorke and Maplestone 1926.

M. yorkei Baylis 1927.

These four species occurred in the stomach of *Macropus agilis* at Burketown. Two further species, apparently belonging to the genus *Macropostrongylus*, from the stomach of *Macropus dorsalis* at Miles, are being investigated.

Cestodes

Fam. HYMENOLEPIDIDAE

Hymenolepis robertsi sp.n. (Fig. 1)

Two specimens of this species occurred, with other Cestodes, in the intestine of a grey teal (*Querquedula gibberifrons*) at Burketown.

Length of strobila about 10–10·5 cm. Maximum width about 2 mm. Diameter of scolex 0·45–0·65 mm. Diameter of suckers about 0·2 mm. Rostellum very large and muscular (seen in the retracted condition only). Hooks 12, about 0·12 mm. long, of the shape shown in Fig. 1.

Number of segments in a complete specimen about 450, of which about 30 are gravid. In a mature segment the ovary is median and has two rounded lateral lobes, between which posteriorly the vitelline gland is situated. Two of the testes are on the aporal side of the ovary, the outer lying slightly anterior to the inner. The third testis is on the poral side. Diameter of testes, when fully

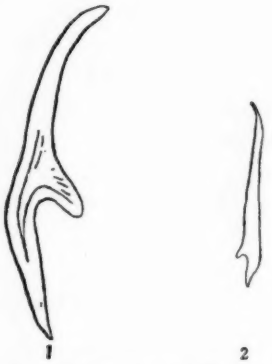

Fig. 1. *Hymenolepis robertsi*. Hook from rostellum.
Fig. 2. *Aploparaksis veitchi*. Hook from rostellum.

developed, about 0·3 mm. Cirrus-sac about 0·6 mm. long and 0·16 mm. in maximum diameter (near its inner end), running obliquely from the genital pore to the anterior margin of the segment, and occupying about one-third of the width of the segment. Cirrus spiny. A spherical external seminal vesicle lies between the inner end of the cirrus-sac and the poral lobe of the ovary. Eggs (measured in balsam): diameter of outer shell, about 0·044–0·048 mm.; diameter of onchosphere, about 0·024–0·028 mm.

The number, size and shape of the hooks in this species appear to be quite distinctive characters. Their number alone seems to be sufficient to separate it from any other form known in Anseriform birds.

Type-specimens in the British Museum (Natural History).

Aploparaksis veitchi sp.n. (Fig. 2)

Several specimens of this species occurred, together with *Diorchis flavescens* and *Hymenolepis robertsi*, in the intestine of the grey teal (*Q. gibberifrons*) at Burketown.

Length of a complete specimen about 40 mm. Maximum width about 0·3 mm. Diameter of scolex 0·17–0·2 mm. Diameter of suckers 0·096–0·106 mm. Rostellum seen in the retracted condition only. Its receptacle projects as a long cone in front of the scolex. Hooks ten, 0·022–0·026 mm. long, of the shape shown in Fig. 2.

Segments very numerous, all very short and relatively wide. The testis, when fully developed, has a maximum diameter of about 0·036 mm. Cirrus-sac about 0·1 mm. long and 0·014 mm. wide, extending to about the middle line of the segment. Cirrus armed with very minute spines, visible only under an oil-immersion objective. A small sub-globular external seminal vesicle lies near the inner end of the cirrus-sac. Eggs sub-globular or slightly ovoid; longest diameter of outer shell 0·035–0·048 mm. Inner shell ovoid, closely investing the onchosphere and measuring 0·022–0·026 × 0·016–0·019 mm.

Tseng (1933) has recently tabulated the species of *Aploparaksis* and given diagrams of the hooks. The majority of species of this genus have hooks with a very short "dorsal" root and a comparatively long "ventral" root. The only species having hooks at all similar in shape to those of the present form are *A. elisae* Skrjabin 1914 and *A. murmanica* Baylis 1919. In *A. elisae* the hooks are of about the same size as in *A. veitchi*, but the worm is much larger, attaining a length of 120 mm. and a width of 1·4 mm., and differs in several other respects, according to Skrjabin's description. In *A. murmanica* the hooks are nearly three times as large as in *A. veitchi*, and the blade is relatively much longer and of a different shape.

Type-specimens in the British Museum (Natural History) and in the Molteno Institute, Cambridge.

Nematode

Fam. TRICHINELLIDAE

Capillaria lophortygis sp.n. (Fig. 3)

Numerous specimens of this species were found by Mr Roberts in the crop of a Californian quail (presumably *Lophortyx californica*) at Brisbane. The bird had been imported from America.

Length, male, 28–30 mm.; female, 37–39 mm. Maximum thickness, male, 0·08–0·09 mm.; female, 0·16–0·17 mm. Cuticle, at least towards the extremities, with fine transverse striations. Bacillary bands present. Length of oesophagus, male, 5·8–6·5 mm.; female, 7·7–8·8 mm. Posterior portion of oesophagus composed of very large cells, which may reach a length of 0·4 mm.

Male: Spicule apparently absent. Spicule-sheath not seen extruded; its hinder portion, in the inverted condition, lined with very minute spines. Two pairs of caudal papillae present—a small preanal and a larger postanal papilla on each side.

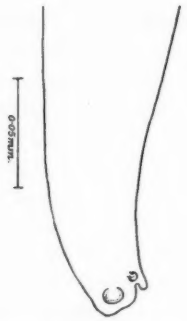

Fig. 3. *Capillaria lophortygis*. Posterior end of male; lateral view, showing papillae.

Female: Rectum 0·2–0·3 mm. long. Vulva at 0·15–0·2 mm. behind junction of oesophagus and intestine; its lips slightly prominent; no eversion of its lining observed. Eggs, when fully formed, very uniform in size (average 0·045 mm. × 0·022 mm.). Shell thick, apparently sparsely punctate.

Capillaria contorta (Creplin 1839) has been recorded by Cram (1931) from *L. californica*. The present specimens cannot be referred to that species, since the males appear to be without a spicule. Of the species of *Capillaria* recorded from Galliform birds only one appears to be without a spicule. This is *C. annulata* (Molin 1858) (synonym, *Trichosomum strumosum* Reibisch 1893), from which the present specimens differ in having no inflation of the cuticle of the head.

Type-specimens in the British Museum (Natural History) and in the Molteno Institute, Cambridge.

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ON THE ANATOMY OF THE TREMATODE *PETASIGER EXAERETUS* DIETZ 1909, FROM THE INTESTINE OF *PHALACROCORAX CARBO*

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(With 2 Figures in the Text)

Introduction

The genus *Petasiger* which belongs to the family Echinostomidae was proposed by Dietz in 1909, with *Petasiger exaeretus* as the type species. The description given by Dietz of this species is incomplete and it is proposed therefore to present below a more detailed account of the anatomy, based on an examination of whole mounts and serial sections. The material was obtained from the small intestine of cormorants shot on the coast of Cardigan Bay between Aberystwyth and Llanrhystyd. Five cormorants were shot in July, and of these two contained a large number of *Petasiger exaeretus* in the small intestine.

The anatomy of *Petasiger exaeretus*

External characters

The body varies from 1·21 to 1·62 mm. in length with a maximum diameter of 0·33 mm. in the region of the ventral sucker. A maximum depth of 0·18 mm. is attained in the region of the shell gland and ovary.

At the anterior end of the body there is a well-developed collar, 0·20 mm. in diameter, bearing 27 oral spines in two alternating rows. Of the four oral spines borne on each lappet the two anterior pairs measure 0·007 mm. and the two posterior 0·004 mm. in length. Towards the dorsal surface of the collar the hooks decrease slightly in size (Fig. 1).

The cuticle is armed dorsally and ventrally with rows of spines (5–7μ long) as far as the ventral sucker.

The oral sucker, 0·07–0·08 mm. in diameter, is situated subterminally with its aperture directed ventrally (Fig. 1). The ventral sucker lies 0·53 mm. behind the oral and is 0·2 mm. in diameter (Figs. 1 and 2).

In the living specimen the pre-acetabular portion becomes arched, the concavity being ventral and the oral sucker opposed to the ventral. In the acetabular region the body is compressed dorso-ventrally and is highly muscular, while in the post-acetabular region the outline is more cylindrical.

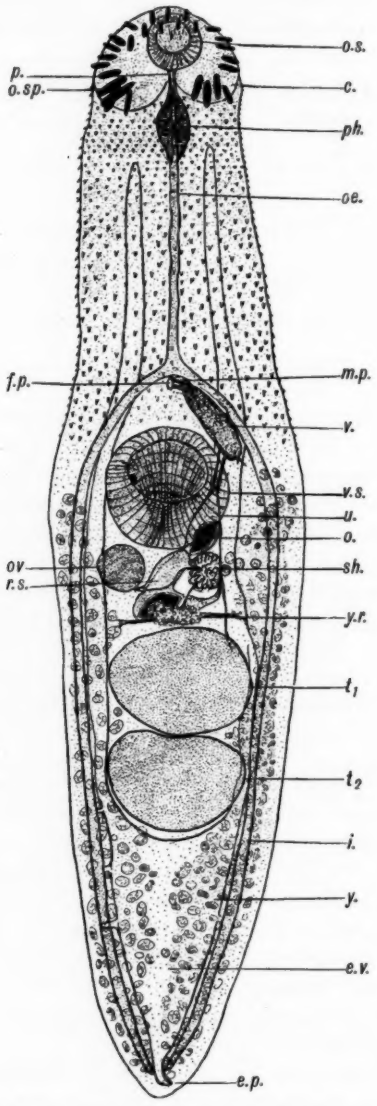

Fig. 1. *Petasiger exaeretus*; general outline.

Lettering: *c.* collar; *c.s.* cirrus sac; *e.p.* excretory pore; *e.v.* excretory vesicle; *f.p.* female genital pore; *i.* intestine; *m.p.* male genital pore; *o.* ovum; *od.* oviduct; *oe.* oesophagus; *o.s.* oral sucker; *o.sp.* oral spines; *ot.* ootype; *ov.* ovary; *p.* prepharynx; *ph.* pharynx; *r.s.* receptaculum seminis; *sh.* shell glands; t_1 anterior testis; t_2 posterior testis; *u.* uterus; *v.* vesicula seminalis; *vg.* vagina; *v.s.* ventral sucker; *y.* yolk glands; *y.d.* yolk duct; *y.r.* yolk reservoir.

Alimentary canal

The mouth opens directly to a prepharynx measuring up to 0·009 mm. in length. The prepharynx opens to a muscular pharynx (Fig. 1).

The pharyngeal cavity is continuous behind with that of the oesophagus. The oesophagus bifurcates a little in front of the ventral sucker, the intestinal rami diverging widely to pass to the posterior region of the body.

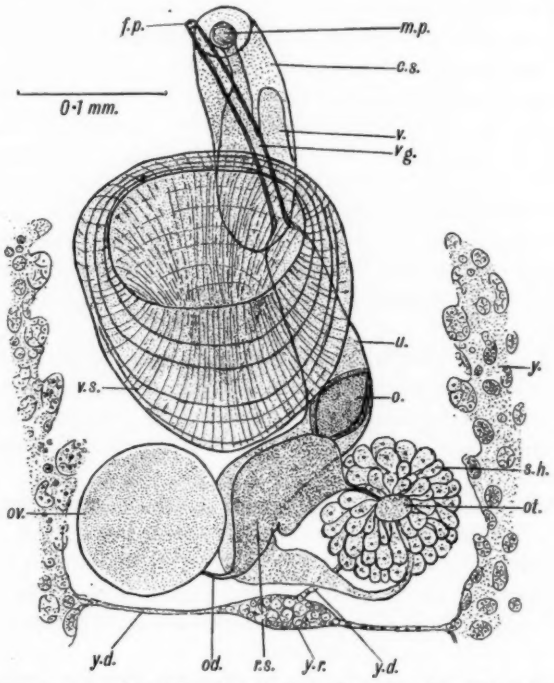

Fig. 2. *Petasiger exaeretus*; male and female genitalia in the region of ventral sucker. Lettering as in Fig. 1.

Excretory system

The excretory pore lies at the posterior extremity. The excretory vesicle consists of an elongated sac extending from the excretory pore to the hinder margin of the posterior testis (Fig. 1). The lumen is very irregular in shape and occupies the greater part of the body between the two intestinal limbs. A few short branches pass dorsal and ventral to the intestinal rami into the vesicle. The vesicle is formed by the union of two lateral excretory vessels (Fig. 1) lying just within the intestinal rami. They occupy a ventral position as far as the ventral sucker where they turn dorso-laterally and cross the alimentary tract on their respective sides. These vessels could be traced only as far as the level of the pharynx.

Genital system

The male and female ducts open separately on the middle line of the ventral surface immediately behind the bifurcation of the intestine.

(i) *Male.* There are two slightly oval testes, 0·22 mm. in their greatest diameter, situated one behind the other in the median line of the post-acetabular region of the body (Fig. 1).

The origin of the vasa deferentia could not be discerned in whole mounts nor in sections. The cirrus pouch which contains the vesicula seminalis is a thin-walled structure extending dorsally and obliquely over the ventral sucker. It is 0·13 mm. by 0·062 mm. (Fig. 2).

(ii) *Female.* The ovary is situated behind the ventral sucker and slightly to the right of the middle line. It is spherical, 0·1 mm. in diameter, and lies a little nearer to the dorsal than to the ventral surface. The oviduct arises from the posterior margin of the ovary, runs for a short distance transversely and then widens to form the receptaculum seminis (Fig. 2) which attains a maximum diameter of 0·075 mm. Distally it diminishes in size and passes through the follicles of the shell gland as a narrow tube to open into the ootype on its right margin.

The shell gland is situated a little to the left of the middle line and midway between the dorsal and ventral surfaces. It is disc-shaped and transversely oval measuring 0·12 mm. in its greatest diameter. It is composed of a number of club-shaped cells and opening into a central ootype. The latter measures 0·067 × 0·04 mm.

The vitelline glands occupy the lateral position of the body. They begin opposite the anterior border of the ventral sucker and extend to the caudal extremity enveloping the alimentary canal on three sides but not overlapping the genital organs. In the post-testicular region they extend inwards and almost unite in middle line. The vitelline glands consist of numerous rounded follicles from 0·02 to 0·04 mm. in diameter.

The walls of the yolk follicles consist of a thin limiting membrane and the components cells are well defined.

The vitelline ducts (Fig. 2) have the usual disposition. The two transverse yolk ducts join to form the yolk reservoir which lies ventrally at the anterior end of the first testis. It is transversely oval in outline measuring 0·08 × 0·04 mm. From this reservoir originates the median vitelline duct which runs forwards, curves to the left, passing through the shell gland to open into the ootype.

The uterus arises from the left side of the ootype. It passes through the follicles of the shell gland and runs dorsal to the receptaculum seminis (Fig. 2). It is a thin-walled duct measuring 0·07 × 0·04 mm. in cross-section with the wall which seems to be composed of three layers. It describes an **S**-shaped bend passing forwards to the ventral sucker where it becomes continuous with the vagina, a thick-walled muscular tube. The vagina runs dorsal to the ventral

sucker and cirrus sac and opens in middle line slightly anterior to the male opening.

The ova are very few in number, the maximum found being nine, but Dietz recorded twenty-six in one specimen. The eggs are oval and measure 0·09 × 0·044 mm.

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A NEW NEMATODE, *LONGIBUCCA LASIURA* N.SP. (RHABDITOIDEA, CYLINDROGASTERIDAE), FROM A BAT

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(With 5 Figures in the Text)

Nematodes of the superfamily Rhabditoidea are usually free-living; however, a few species appear to be definitely parasitic. The Nematode described in this paper belongs to the latter group. It was found in large numbers in the small intestine of one of two bats, *Lasiurus borealis*, captured September 15th, 1932, in the vicinity of Washington, D.C. Additional specimens of this species of bat were not obtained for examination. Two specimens of each of two other species of bats, *Eptesicus fuscus* and *Nycticeius humeralis*, collected from the same locality were negative for this parasite. The Nematode is a member of the family Cylindrogasteridae Chitwood 1933, and appears to be congeneric with *Longibucca vivipara* Chitwood 1933, found in the wall of the stomach of a South American snake, *Pseudoboa cloelia*.

Longibucca lasiura n.sp.

Specific description. Longibucca: Oral opening surrounded by six small discrete lips. Cephalic papillae consisting of an internal circle of six well-developed papillae and an external circle of six papillae of which four (laterodorsals and lateroventrals) are well developed and two (ventrolaterals) are reduced. Amphids dorsolateral, pore-like (Fig. 1). Buccal cavity 40–44 μ long in male and 47–50 μ long in female, terminating in small cuticular thickenings (Figs. 2 and 5). Esophagus 56–60 μ long, consisting of a subcylindrical corpus, approximately 22–32 μ long by 7–8 μ wide, indistinctly set off from isthmus and pseudobulb. Distance from anterior extremity to base of esophagus, 97–120 μ. Ratio of length of buccal cavity to length of esophagus, approximately 5 : 6. Nerve ring 68–78 μ from anterior end of body. Excretory pore 100–105 μ from anterior end.

Male 660–740 μ long by 22–28 μ wide. Anus 78–93 μ from posterior extremity; tail conically elongate. Testis extending to within 300–400 μ of anterior end of body. Genital papillae: one pair of preanal subventral and three pairs of postanal subventral, and one pair of postanal subdorsal. Spicules unequal;

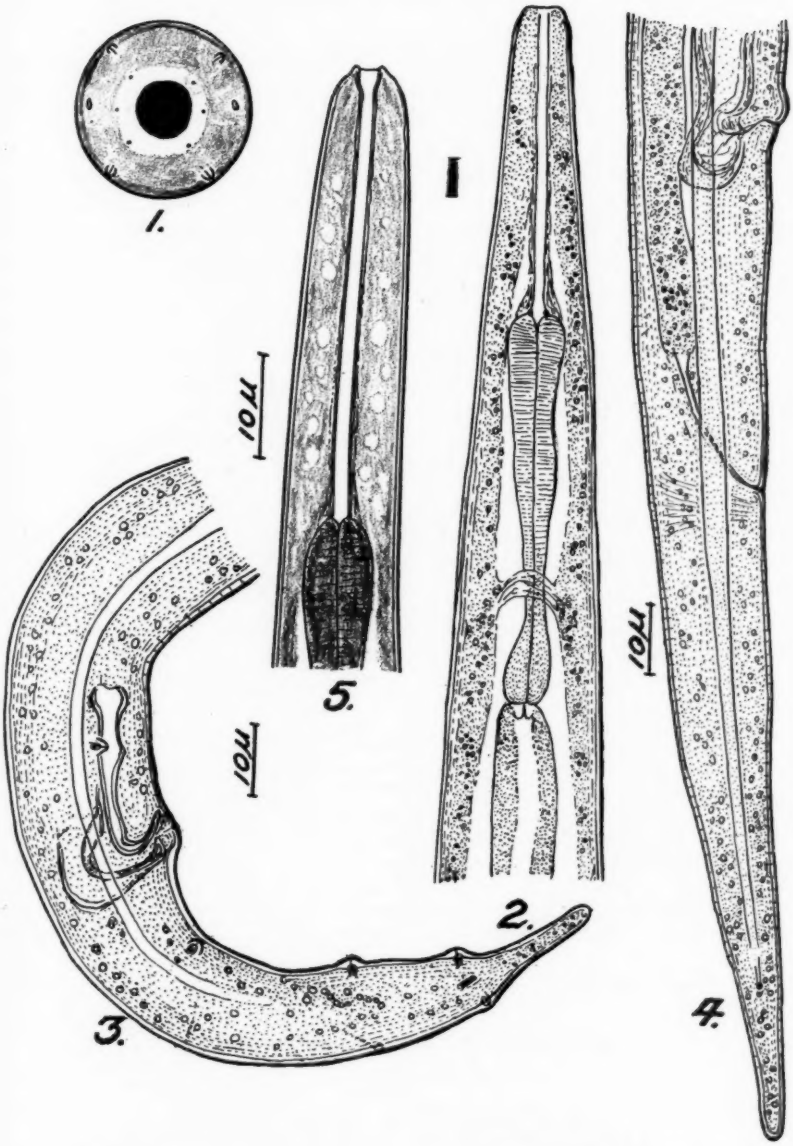

Fig. 1. *En face* view.
Fig. 2. Esophageal region, dorsal view.
Fig. 3. Tail of male, lateral view.
Fig. 4. Tail of female, lateral view.
Fig. 5. Cephalic region, dorsal view.

large spicule 29–31 μ long, hook-like, heavily cuticularised; small spicule thin, arcuate, 22–25 μ long, apparently fused distally with gubernaculum. Gubernaculum 16–20 μ long (Fig. 3).

Female. 680–840 μ long by 30–44 μ wide. Anus 70–87 μ from posterior extremity; tail conically attenuated, not curved dorsad. Ovary extending to within 200–260 μ of anterior extremity. Vulva 560–690 μ (71–87 per cent. of total body length) from anterior extremity. Distance from vulva to anus, 50–52 μ. Viviparous; one larva or two larvae in uterus. Egg shell stretching with growth of larva and sometimes reaching a length of 250 μ, distending the uterus, the uterus and its contained egg almost completely filling the body cavity; shell finally breaking open at hatching of larva.

Host. Lasiurus borealis (Müller).

Location. Small intestine.

Distribution. United States (Washington, D.C.).

Type specimens. United States National Museum Helminthological Collection No. 33,041; paratypes, No. 33,043.

Differential diagnosis. Longibucca lasiura differs from *L. vivipara*, the only other species of the genus, in the following respects: The corpus is well set off from the isthmus in *L. vivipara* and indistinctly set off in *L. lasiura*; the spicules of the male are equal in the former species and unequal in the latter; and both the male and female tails differ in their contours in the two species (Figs. 3 and 4)[1].

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CHITWOOD, B. G. (1933). Some Nematodes of the superfamily Rhabditoidea as parasites of reptiles and amphibians. *J. Wash. Acad. Science*, **23** (11), 508–20, figs. 1–20.

[1] Note added 21. XII. 1933. Additional specimens of *Longibucca lasiura* were obtained from the big brown bat, *Eptesicus fuscus fuscus* (Beauvois), collected in Washington, D.C., November 2, 1933. Approximately 30 specimens were found in the stomach which was afterwards fixed in formalin and sectioned. Examination of the sections showed that several adults were present in a layer of mucus and desquamated epithelium; this layer also contained lymphocytes and polymorphonuclear leucocytes. Several adult and larval worms were also found embedded deep in the mucosa, especially in the depths of the glands and in the interglandular tissue; a few worms were found with the anterior end in the mucosa and the posterior end in the lumen. The tissue of the stomach showed no marked pathological reaction.

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ON THE AMPHILAPHORCHIDINAE, A NEW SUBFAMILY OF PROTEOCEPHALID CESTODES, AND *MYZOPHORUS ADMONTICELLIA*, GEN. ET SP.N., PARASITIC IN *PIRINAMPUS* SPP. FROM THE AMAZON

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(With Plate III, containing Figs. 1–14, and Fig. I in the Text)

Amphilaphorchis piranabu, gen. et sp.n. from **Pirinampus pirinampus** (Spix)

"Piranabú," "Piranambú" or "Piranampú" (the last according to Goeldi, 1898) are local names on the Amazon applied to the siluroid fish which, so far as it is possible to determine from memory and from the rough sketches and notes which I made at the time, is the modern *Pirinampus pirinampus* (Spix). This fish, of which I examined eight examples, attains a length of at least 60 cm., has an elongated adipose fin more than one-third the length of the entire body, microscopic scales, possesses maxillary barbels about half the length of the body, and in general appearance closely resembles the figure (Tab. VIII, *a*) of "*Pimelodus ctenodus*" provided by Spix (1829). I am much indebted to Mr J. R. Norman, of the British Museum, for his kind assistance in identification.

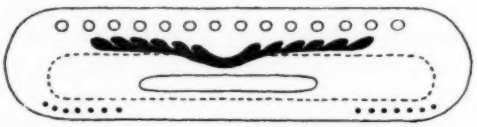
In the middle and hind intestine of one example of the Piranabú, purchased in the market at Manáos, I found eight unattached specimens of a tapeworm which, though insignificant in size, is yet remarkable as representing a new grade of structure in the family of the Proteocephalidae. My largest specimen was approximately 10 mm. in length, with a maximum breadth (posteriorly) of 0·88 mm. The strobila is fairly uniform in width, and the total number of segments is probably less than fifty. The anterior segments extend up to the base of the scolex and are very short (roughly thirty in number) and next to the scolex are 0·46–0·58 mm. wide, but these soon lengthen and widen and the greater part of the strobila length consists of gravid segments, the four or five hindermost being either square in outline (Fig. 3) or longer than broad (*e.g.* 1·24 mm. long and 0·66 mm. broad). The cirro-vaginal apertures are marginal and irregularly alternate and are each situated a short (variable) distance in front of the middle point of the proglottid length. The uterus pore is formed as usual by the ventral uterus wall coalescing with the ventral subcuticula. The scolex (Figs. 1, 2) is of the massive wrinkled type, not uncommonly found in these aberrant Proteocephalids of Siluroids (*e.g.* in species of *Goezeella*, *Ephedro-*

cephalus, *Peltidocotyle*, *Amphoteromorphus* and *Othinoscolex*). It measures 0·58–0·68 mm. in breadth and 0·29–0·49 mm. in height. The sides of the thick column bear many longitudinal furrows and the end is truncated, with a prominence in the centre, at the edges of which are to be found the openings of four conspicuous suckers (external diameter of the entire sucker in vertical sections of one example, 0·24 mm.). Hooks and spines are entirely absent.

The structure of a gravid proglottid (Fig. 3) is as follows. The testes are roughly about a hundred in number (possibly more) and are disposed in a single field, one layer deep, over almost the entire area of the proglottid in the dorsal cortex. Even in the region of the ovary they are present along the margins. One striking peculiarity of the testes in this species is the fact that they extend not only to the very edges of the proglottid but also extend ventrally at the edges so as to lie level with or even ventral to the ventral excretory canals; in other words, the sheet of testes bends ventrally at the margins (Fig. 4). The name of the new genus—*Amphilaphorchis*—is founded on this peculiar distribution of the testes. The testes in transverse sections measure about 0·073 × 0·054 mm. The cirrus sac, in a whole preparation (Fig. 3), measures 0·16 mm. long and 0·058 mm. broad, with the unarmed cirrus extruded (0·065 mm. long). The vas deferens forms a small mass of coils on leaving the sac. The vagina is slightly dilated next to its opening which is apparently behind the cirrus sac, and it joins the ovary in the usual manner. The ovary is a mass of large follicles which extends over a considerable area (Fig. 3) and is of interest in that the majority of the follicles lie in the cortex, *i.e.* outside the layer of longitudinal muscle fibres, though a number of central more deeply situated follicles remain medullary (Fig. 4). The ovary is thus to some extent in the condition which has been described by La Rue (1914) for *Monticellia coryphicephala*, in which "dorsal prominences and the greater part of the ovarian lobes from which they arise lie in the cortical parenchyma outside of the internal layer of longitudinal muscles. The mid-piece and part of the lobes of the ovary lie within the muscular sheath"; in the present species apparently a greater proportion of the ovary is medullary. The uterus is large and bears relatively short bulky diverticula which in all my specimens are filled with eggs. A wide uterine "duct" is present, dorsal to the ovary, which opens into the uterine sac at the anterior end of the ovary. It is remarkable that while the ovary is largely cortical the uterus is entirely medullary. The intra-uterine eggs (drawn in the original formalin-glycerine preserving fluid) are oval in form, the outer shell having a distinct opening at each end (Fig. 5). In a few eggs the terminal openings appear to be closed up by a plug which may carry a fine filament. The outer shells measure about 26 × 20 microns and the hookless embryo 10–11 microns in diameter. The vitellaria appear to be very shapeless in my material, and I was quite unable to ascertain the size of individual vitellaria. They form, in transverse sections, two short marginal rows, each situated ventral to the ventral excretory canal. The usual two pairs of excretory canals are present, the dorsal being very minute. The layer of longi-

tudinal muscle fibres has the arrangement shown in Fig. 4. The fibres are few in number and not aggregated into bundles, and careful staining is necessary (with eosin or some special stain) in order to ascertain their situation relative to the principal organs. The parenchyma is free from calcareous corpuscles.

I have recently (Woodland, 1933*a*, 1933*c*) proposed the subdivision of the Proteocephalidae into six subfamilies on the basis of the various relationships of the principal genital organs to the internal longitudinal muscle layer. These six subfamilies are the Proteocephalinae Mola 1929, Zygobothriinae Woodland 1933, Marsypocephalinae Woodland 1933, Ephedrocephalinae Mola 1929, Othinoscolecinae Woodland 1933 and Monticelliinae Mola 1929, and they are defined in my recent paper in this *Journal* (Woodland, 1933*c*). I realised, however, that there was a possibility of the existence of at least one more subfamily in the form of an alternative to the subfamily Othinoscolecinae. In this last subfamily the testes, vitellaria and uterus are cortical in position, the ovary alone (apart of course from the cirrus sac, vas deferens and vagina) being medullary; the alternative would be for the ovary to be cortical and the uterus to be medullary. This alternative hypothetical condition was found by me to be an actual fact in the very next Cestode which I examined and which is



Text-fig. I. Amphilaphorchidinae

described above. This Cestode I propose to name *Amphilaphorchis piranabu*, with reference to the curious distribution of the testes and the local name of the host. The new subfamily which I suggest in order to accommodate this seventh condition of the principal genital organs relative to the longitudinal muscle layer must be called the Amphilaphorchidinae, and may be defined as follows: Proteocephalidae in which the testes and vitellaria are cortical and the ovary entirely or partly cortical, only the uterus being wholly medullary. The vitellaria are dispersed and are ventral or marginal in position. The new genus *Amphilaphorchis* may provisionally be defined as follows: Amphilaphorchidinae. Scolex of massive form, bearing suckers and devoid of apical organ. Marginal cirro-vaginal pores irregularly alternate and the vagina opens behind the cirrus sac. The testes field is broader than the containing segment, the testes extending to the extreme margins and thence ventrally so that the edges of the field are folded. Vitellaria lateral and ventral.

In my description of the structure of *Peltidocotyle rugosa* (Woodland, 1933*a*) it was stated that the specimens were so immature that it was impossible to describe the uterus and the species was provisionally referred to the Monticelliinae, assuming that the developed uterus, like the testes, vitellaria and

ovary, would be found to be cortical in position. The existence of *Amphilaphorchis piranabu*, however, shows that this assumption is not necessarily valid, and it is possible and indeed seems probable that when gravid segments of *Peltidocotyle rugosa* come to be examined, this species will have to be referred to the Amphilaphorchidinae rather than to the Monticelliinae.

Amphilaphorchis myoides sp.n.

In a Piranabú caught seventy miles below Manáos I found three small Cestodes, the longest measuring about 11 mm. in length and with a maximum breadth of about 0·6 mm., which proved to be a well-defined second species of *Amphilaphorchis*. The strobila is, as in *Amphilaphorchis piranabu*, fairly uniform in width, with externally ill-defined and somewhat wrinkled segments probably less than fifty in total number, the majority of which are gravid. So far as I can ascertain from external examination only the penultimate and end segments are longer than broad (0·17 mm. long and 0·61 mm. broad in one specimen), all the preceding being broader than long. The cirro-vaginal apertures are irregularly alternate and are each situated a short distance in front of the middle point of the proglottid length. Uterine pores are not found in any of the segments I have examined. The scolex is, as in *Amphilaphorchis piranabu*, of the massive type (Fig. 6), with a wrinkled surface and a slight apical projection beyond the four suckers. The unarmed scolex, in my largest specimen, is 0·59 mm. broad and about 0·47 mm. long, but externally there is no definite line of junction between scolex and strobila. Viewed as a transparent object, however, the scolex is limited posteriorly by the segmentation of the strobila which extends up to the scolex base. So far the description of the present species is practically identical with that of the type species *Amphilaphorchis piranabu*, the differences being indefinable, and indeed the proglottid structure is also formed on the same plan and it is only two (or three) features in this latter—fortunately both very distinct—which enable the two species to be clearly differentiated.

The structure of a gravid proglottid is as follows. The testes, as in *Amphilaphorchis piranabu*, are (at a very rough estimate) about one hundred in number, disposed in a single field one layer deep over the whole of the dorsal cortex anterior to the ovary (not lateral to the ovary as in *Amphilaphorchis piranabu*), and, as in *Amphilaphorchis piranabu*, this layer of testes is curved ventrally at the margins (Fig. 7). In transverse sections the testes measure about 0·051 × 0·033 mm. and are therefore distinctly smaller than in *Amphilaphorchis piranabu*. The cirrus sac, in a transverse section, measures 0·237 × 0·047 mm. and is thin-walled. The unarmed cirrus itself is not coiled in the sac, but several coils of the vas are visible in the inner end of the sac and, external to the sac, the vas forms the usual convoluted mass. The vagina, differing from that in *Amphilaphorchis piranabu*, opens anteriorly to the cirrus sac, and its portion next the opening is slightly dilated and lies ventral to the sac. The ovary (Fig. 8) is of the same type as that described for *Amphilaphorchis piranabu*,

though the two halves where they emerge, through the layer of longitudinal muscle bundles, into the cortex, are even more widely separated than they are in that species. The uterus, in my material, is not large, possesses no extensive lateral diverticula and has not formed a uterine pore. As in *Amphilaphorchis piranabu*, the uterus is entirely medullary. The intra-uterine eggs are very distinctive in form (Fig. 9), the outer shell being drawn out at each end. The eggs measure 62–77 microns in length, the breadth of the middle region being about 16·4 microns. The hookless embryo is 9–10 microns in diameter. The vitellaria are individually about a quarter of the diameter of the testes and are disposed in two short rows ventral to the lateral edges of the sheet of longitudinal muscle fibres. The second distinctive feature of the present species is the relatively marked development of the longitudinal muscle-fibre system. In *Amphilaphorchis piranabu* the sheet merely consists of a very attenuated layer of individual fibres and its course in transverse sections is not conspicuous, but in the present species the "sheet" consists of numerous fibres clustered into large, closely packed and fairly definite bundles and is very conspicuous. Large ventral excretory canals are present, but I have not detected dorsal canals.

Apart from the shape of the eggs and the greater development of the longitudinal muscle system, *Amphilaphorchis myoides* only differs appreciably from the type species in the position of the external end of the vagina relative to the cirrus sac (posterior in the latter and anterior in the former), in the absence of testes at the sides of the ovary and in the smaller size of the testes.

Myzophorus admonticellia, gen. et sp.n. from **Pirinampus** spp.

In a second specimen of the Piranabú caught at Manáos, in a third caught in a backwater near Urucará (between Itacoatiara and Parintins) and in another fish, caught ten miles below Itacoatiara and named "Pēracachinga da grandje" by the Indian fishermen (this last fish closely resembled the figure of "*Pimelodus piranampu*" provided by Spix and Agassiz, 1829, on their Plate VIII and is obviously nearly related to *Pirinampus pirinampus*, though differing in possessing much thicker barbels: it is probably *Pirinampus typus*), I found in the intestines numerous examples of the tapeworm now to be described. The largest of these worms measured about 100 mm. in length, with a maximum breadth of only a little over 1 mm., the strobila being very uniform in breadth. The anterior segments are very short and are restricted to the relatively short narrow region (about one-fifth the length of the strobila in one worm) immediately behind the scolex; more posterior segments soon become mature and approach the square in outline, and the greater part of the length of the strobila (about two-thirds) is composed of gravid segments all longer than broad. One of the longest segments measured 0·88 mm. in breadth and 6·1 mm. in length. The end proglottid only differs from those preceding in possessing a rounded posterior edge. The marginal cirro-vaginal apertures are irregularly alternate and are each situated at about a third of the proglottid length from the anterior end. Uterine pores are not formed in my material, though they are without

doubt of the usual ventral slit type. All the uteri are filled with eggs. The unarmed scolex (Fig. 10) is of simple form, consisting of a central pillar ending in a rounded prominence and bearing four ordinary suckers near the extremity. The size of the scolex varies considerably, the breadth being 0·249–0·48 mm. and the height 0·165–0·36 mm., and the maximum external diameter of the suckers is, according to absolute size and state of contraction, 0·149–0·265 mm. Occasionally the base of the scolex is surrounded by a few transverse folds forming a kind of (temporary) collar, but these do not represent segments. The unsegmented region of neck varies in length between 0·16 mm. and 1·32 mm. and in breadth 0·099–0·43 mm., according to state of contraction and point of origin of segmentation.

The structure of a gravid proglottid (Fig. 11) is as follows. The testes occupy a single field (thin in the middle line) bounded laterally by the vitellaria and posteriorly by the ovary and are roughly about two hundred in number. In surface view the testes measure individually about 0·047 mm. in diameter. The cirrus sac is large and (with the cirrus retracted) measures in surface view about 0·33 mm. in length and 0·149–0·182 mm. in breadth, and contains, besides the cirrus, some coils of the ductus. Externally to the sac the vas forms a large and distinct mass of coils, situated internal and posterior to the sac. The vagina is somewhat dilated in the region of the sac and opens anteriorly to it; posteriorly it runs in the middle line direct to the ovary without any convolutions. The ovary in surface view appears as two triangular lateral areas united by a thin median bridge posteriorly. In transverse sections (Fig. 12) the ovary is seen to be strictly unilaminar and to bear a number of projections on its dorsal surface, so that the mass of the organ is more dorsal than ventral, and indeed this form of the ovary strongly reminds us of those described by La Rue in *Monticellia coryphicephala*, in *Amphilaphorchis* spp. described above and in some other Proteocephalid species, in which the organ is wholly or in part situated in the dorsal cortex. The uterus is large and bears numerous lateral diverticula, filled with eggs which are retained there until the uterine pore is formed at a very late stage of development. In the older gravid proglottids the uterine diverticula become larger and more separated from each other than is shown in Fig. 11. The intra-uterine eggs (drawn in the original preserving fluid) are somewhat irregular in form and of the appearance shown in Fig. 14. The diameter of the outer irregular covering is about 30 microns on the average, and of the inner thick shell about 22 microns, and of the unarmed embryo 11–12 microns. The vitellaria are numerous, about half the average diameter of the testes (in transverse sections) and, in transverse sections, are arranged in the form of two marginal crescents. The usual two lateral excretory canals are present on each side, the ventral varying greatly in size according to distension. The longitudinal muscle system is distinctly of the Phyllobothriid type, *i.e.* it consists of fibres and (internally) ill-defined bundles of fibres scattered, in transverse sections, throughout a zone extending from underneath the cuticle to a certain depth in the parenchyma (in this case about one-third of the

thickness of the proglottid), and yet it possesses several special features which are of interest because, like the scolex suckers, they appear to signify an approach to the Proteocephalid type. These special features, I must add, are only to be seen in sections of proglottids which are contracted and in which therefore the muscle-fibres are especially thick and separated from each other transversely; in extended proglottids the fibres are too thin and crowded together to show their true characteristics. The most important of these special features is (Figs. 12, 13) the separation-off of an innermost layer of especially large fibres from the general zone of fibres, the separation being effected by the appearance of an actual gap between them and the more external fibres and by the fibres of this innermost layer being larger and becoming clustered into ill-defined bundles not found elsewhere. This innermost layer bears a distinct resemblance to the layer of bundles characteristic of most Proteocephalids. Another feature is that this internal layer of bundles is situated well inside the internal limits of the subcuticula, so again resembling the Proteocephalid layer and differing from the longitudinal musculature of most Phyllobothriids. Finally the innermost layer of bundles lies internal to the crescents of vitellaria and to the uterus and to the outer ends of the dorsal projections of the *Monticellia*-like ovary, so that, in the case of a Proteocephalid, these organs or portions of organs would have to be regarded as cortical in position; on the other hand, the testes lie wholly internal to the bundle layer (and are more centrally situated than the dorsally placed ovary) and would in consequence have to be regarded as medullary. It so happens, however, that in no known Proteocephalid have the organs collectively this particular arrangement relative to the muscle layer, the testes being, with the sole exception of the vitellaria, the first organs to become cortical in position, and only in the Zygobothriinae have the testes remained medullary while other organs (the vitellaria) have become cortical (Fig. I). I name this species *Myzophorus admonticellia*, the specific name having reference to the quasi *Monticellia*-like form of the ovary and to the peculiar disposition of the innermost longitudinal muscle-fibres relative to the organs named.

Despite the possession of typical proteocephalid suckers, a unilaminate ovary and the presence of an almost proteocephalid-like layer of muscle bundles, this worm must, on account of the general distribution of the longitudinal muscle fibres, the crescentic arrangement of the vitellaria and the late development of the uterine pore, be referred to the Phyllobothriidae. In other words, it belongs to the same group as the two species of "*Anthobothrium*"—*Anthobothrium piramutab* and *Anthobothrium karuatayi*—which I have described elsewhere (Woodland, 1933*b*, 1934*a*), this group differing from typical Phyllobothriids in possessing a unilaminate ovary and in being parasitic in fresh-water Siluroids. Further, the present species differs from the two species of *Anthobothrium* just named in possessing a scolex with typical Proteocephalid suckers. This being the case, it cannot possibly be referred to the genus *Anthobothrium*. The only Phyllobothriid so far known to possess sucker-like organs is the

Anthobothrium giganteum of van Beneden, on which character I founded (Woodland, 1927) a new genus for the species, viz. *Scyphophyllidium giganteum*. But the present species cannot justly be referred to *Scyphophyllidium*, if only because in the one known species of this genus the proportion of gravid proglottids to immature is probably very small (in one specimen 9 cm. of the strobila still only showed immature proglottids), whereas the reverse condition is strikingly illustrated in the present species; further the suckers of *Scyphophyllidium giganteum* are not typical, the ovary is quadripartite and the host is a marine Elasmobranch. The only course to pursue is to create a new genus, *Myzophorus*, in the Phyllobothriidae, the leading characters of which are the possession of typical suckers, a strobila in which, when sexually mature, gravid proglottids compose at least half of the strobila, a unilaminate ovary and the being parasitic in a fresh-water Siluroid.

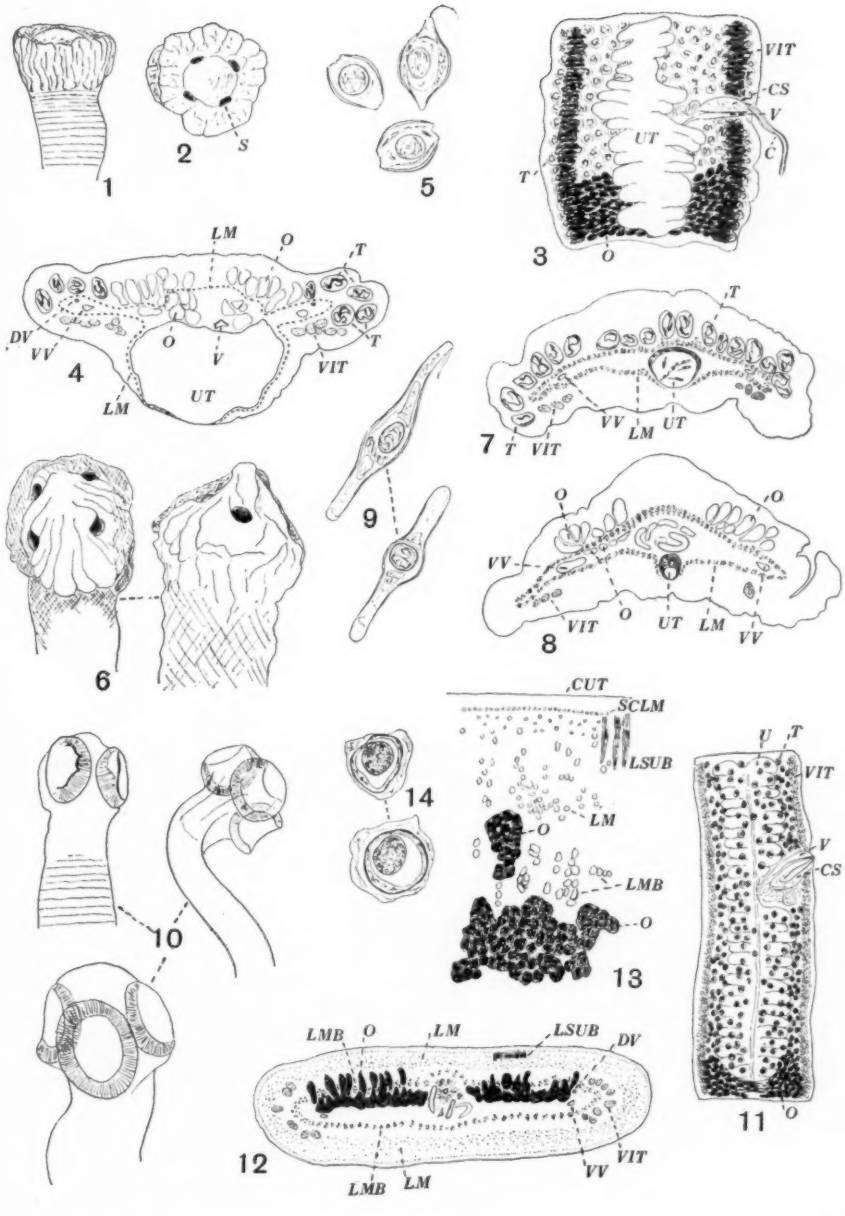
I may mention in conclusion that I was at first of the opinion that the worms found in the two Piranabú and the large- and small-headed worms found in the Peracachinga represented three distinct subspecies, but in the absence of more material I cannot come to a decision.

I wish to thank Miss I. M. Bellis of the Wellcome Research Institution for her kind assistance in translating from the Greek.

NOTE ADDED 23. I. 1934, concerning **Peltidocotyle rugosa** Diesing

Professor Fuhrmann, who has recently examined the type specimens of *Peltidocotyle rugosa*, says in a private letter to me that these have a scolex resembling that of the species which I, with some doubt, described under the name of *Peltidocotyle rugosa* (Woodland, 1933*a*—Fig. 31) but with four distinct suckers "similar to those of *Amphoteromorphous*" and that the ovary is medullary, and, after reading my description of *Othinoscolex* (Woodland, 1933*c*), he concludes that "*Othinoscolex* is synonymous with *Peltidocotyle* Diesing" (my species *Othinoscolex myzofer* bears suckers similar to those of the true *Peltidocotyle rugosa*, but apparently the two species are not identical). This being the case, the name Othinoscolecinae must give place to Peltidocotylinae, as suggested by Professor Fuhrmann in his letter, and the species which I described as *Peltidocotyle rugosa* will probably have to be referred to the Amphilaphorchidinae, as I have stated above in the text. This species cannot be regarded as a third species of *Amphilaphorchis* because it lacks the characteristic overlapping at the margins of the wide sheet of testes and has marginal and crescentic vitellaria. It appears therefore to be necessary to institute a new genus for its reception and I suggest the name *Spatulifer*.

This new genus *Spatulifer* may provisionally be defined as follows: Amphilaphorchidinae. Scolex of massive form, with spatulate (adhesive?) areas, or suckers, or suckerless, and devoid of apical organ. Marginal cirro-vaginal pores irregularly alternate and the vagina opens behind the cirrus sac. The testes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field is of normal extent. Vitellaria marginal and crescentic (in transverse sections).

The specific name I suggest is *surubim*, after the native name of the host: *Spatulifer surubim*.

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EXPLANATION OF PLATE III

Amphilaphorchis piranabu, gen. et sp.n. (Figs. 1–5)

Fig. 1 (×27): the scolex in side view.

Fig. 2 (×27): end-on view of scolex.

Fig. 3 (×39): ventral aspect of gravid proglottid. The numerous eggs in the uterus are not shown.

Fig. 4 (×56): transverse section through gravid proglottid in region of ovary. The position only of the longitudinal muscle-fibres is indicated by the dotted line. This section also shows the ventral extension of the testes at the margins.

Fig. 5 (×395): intra-uterine eggs as seen in formalin.

Amphilaphorchis myoides, sp.n. (Figs. 6–9)

Fig. 6 (×39): the scolex in end-on and side views.

Fig. 7 (×87): transverse section across young gravid proglottid anterior to ovary.

Fig. 8 (×87): transverse section through similar proglottid in region of ovary.

Fig. 9 (×395): intra-uterine eggs of peculiar elongated form sketched in formalin.

Myzophorus admonticellia, gen. et sp.n. (Figs. 10–14)

Fig. 10 (×56): three forms of the scolex.

Fig. 11 (×17): dorsal aspect of gravid proglottid.

Fig. 12 (×56): transverse section through a contracted gravid proglottid to show the peculiar distribution of the internal longitudinal muscle-fibres and the form and position of the ovary.

Fig. 13 (×250): small portion of the section of Fig. 12 to show the position and size of the longitudinal muscle-fibres in the parenchyma and relative to the ovary.

Fig. 14 (×395): intra-uterine eggs drawn in formalin.

Lettering to figures. *C*, cirrus; *CS*, cirrus sac; *CUT*, cuticle; *DV*, dorsal excretory canal; *LM*, longitudinal muscles; *LMB*, longitudinal muscle bundles; *LSUB*, limit of subcuticular layer; *O*, ovary; *S*, sucker; *SCLM*, subcuticular layer of longitudinal muscle fibres; *SUB*, subcuticular layer; *T*, testes; *UT*, uterus; *V*, vagina; *VIT*, vitellaria; *VV*, ventral excretory canal.

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TURNING SICKNESS AND EAST COAST FEVER

PRELIMINARY COMMUNICATION

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(With Plate IV)

Turning sickness of cattle, a very fatal disease, long known in tropical East Africa, is characterised by circling or turning movements made by the affected animals. Recent observations prove that the malady occurs sporadically throughout Uganda and in the greater part of Kenya and Tanganyika where East Coast Fever is highly enzootic. It appears to occur mostly in hot dry periods of the year.

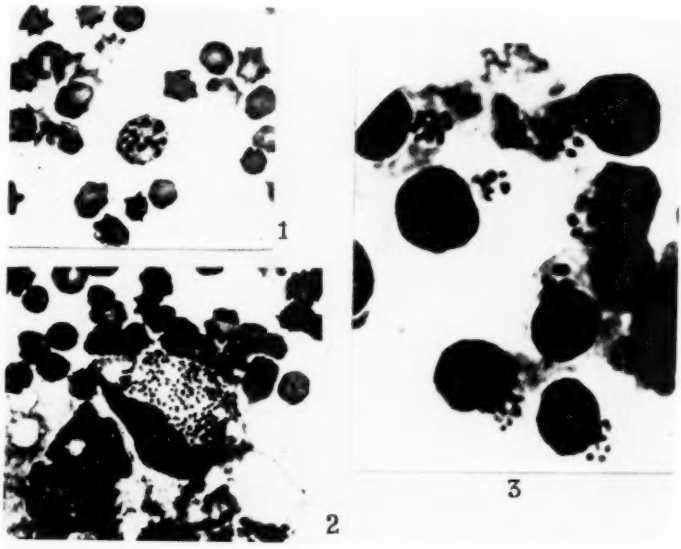
The natives have ascribed the disease to an undetermined poisonous plant, but experiments with various plants have proved negative.

Case I. In January, 1932, a *typical case* was brought to our laboratory at Entebbe. The animal was killed when moribund. Its brain showed remarkable macroscopic lesions: acutely engorged capillaries; a florin-sized haemorrhage over the posterior lobe of the left hemisphere; scattered haemorrhages, usually small, punctiform, clearly defined, were found scattered throughout the brain; they varied in size; one large extravasation (4 × 2·75 cm.) had seriously disorganised the nervous tissue of the posterior pole of the hemisphere.

Films from the haemorrhages, stained by Giemsa, showed what appeared to be typical "Koch's blue bodies." Formalin-fixed sections showed schizonts in medium sized or large lymphocytes crowding the brain capillaries in vast numbers; some capillaries and even arterioles were occluded by emboli of schizont-laden lymphocytes. Films from the spleen, lymphatic glands, etc., showed no "Koch's bodies," while *Theileria* were rare in the peripheral blood. Such appearances are not observed in typical East Coast Fever or different kinds of Theileriasis where the schizonts are plentiful in the spleen and lymphatics and rare or absent in the brain.

On tracing the history of the case it was found that the animal was born and bred in an enzootic East Coast Fever area, and two years before had had this disease and apparently recovered completely.

Cases II–XII. Subsequently eleven other cases were observed at Entebbe: all had previously had East Coast Fever severely or slightly. The turning movements followed at a variable interval of time after recovery from East Coast Fever, i.e. 2 months in a 5½-months calf, up to 5 years in a 6-year-old cow. All the animals had been continuously exposed to tick infestation in highly enzootic country.



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Clinically, the symptoms are not always patent and they may be absent. In such cases the nature of the disease is revealed at autopsy and by microscopic examination as in Case I. In ten animals the disease lasted, on an average, 4·3 days, two survived 21 and 54 days respectively.

DISCUSSION

Whilst leaving further details concerning Turning Sickness to be dealt with in another paper, it may be noted that the relationship between it and East Coast Fever requires further elucidation. Hitherto it has been widely held that life-long immunity follows on recovery from East Coast Fever. A second attack is rare and where it occurs it is regarded as due to reinfection, but in no recorded cases of supposed reinfection have effects been observed like those herein described.

The possibility exists that Turning Sickness may be due to a malignant strain of *Theileria parva*. In some respects the microscopical features recall what is observed in cerebral malaria of man where the brain capillaries are clogged with developing *Plasmodium falciparum*.

ACKNOWLEDGMENTS. I am indebted to the following gentlemen for help: Mr W. F. Poulton, Director of Veterinary Services, Uganda Protectorate, for furnishing cases for study and for permission to publish this note; Mr R. Daubney, Director of Veterinary Research, Kabete, Kenya, for preparing the photo-micrographs serving as illustrations; Prof. Nuttall, F.R.S., Prof. Keilin, F.R.S., and Dr C. W. Wenyon, F.R.S., for friendly counsel.

EXPLANATION OF PLATE IV

Fig. 1. Free agamont in smear from cerebral vessels of heifer X 9158. Kabete case. (×1100.)
Fig. 2. Gamont in lymphocyte. Smear from cerebral vessels of heifer X 9158. Kabete case. (×1100.)
Fig. 3. Lymphocytes containing agamonts. Section of capillary in cerebral cortex of case 325. (×2200.)

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NOTICE

A CENTRAL MALARIA LIBRARY

A Malaria Library was founded in Rome by the Stazione Sperimentale per la Lotta Antimalarica in 1925, and an *Index to Malaria Literature* is issued annually by the Station.

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